AD-A122 728 ANNUAL RESEARCH PROGRESS REPORT FY 1980(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA J D MARSHALL 01 OCT 80 1/3

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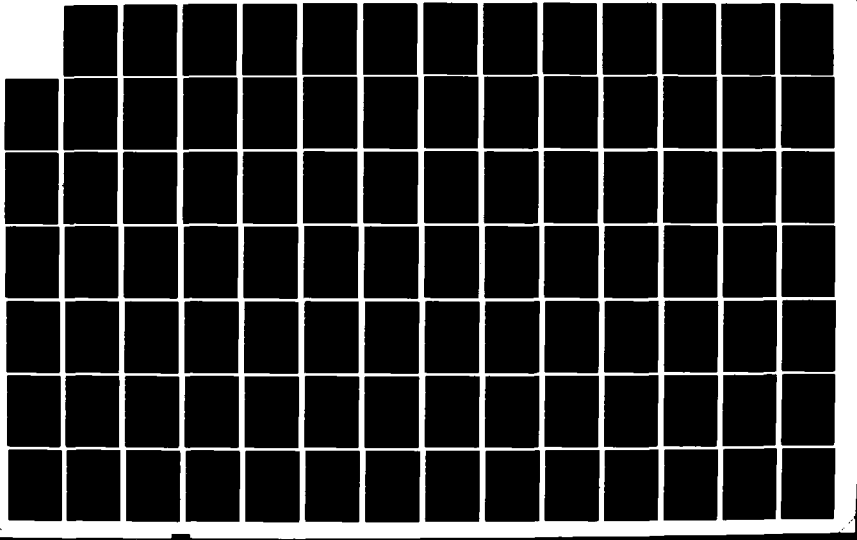

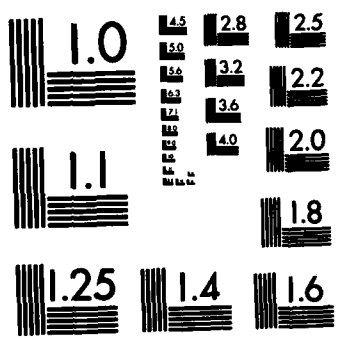

(82) (11)

LETTERMAN ARMY INSTITUTE OF RESEARCH
ANNUAL RESEARCH PROGRESS REPORT

FY 1980

RCS-MEDDH-288(R1)

30 SEPTEMBER 1980

LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER RCS-MEDDH-~~288~~(R1)	2. GOVT ACCESSION NO. AD-A122728	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* Letterman Army Institute of Research, Annual Progress Report, FY 1980		5. TYPE OF REPORT & PERIOD COVERED Annual Research Progress Report, 1 Oct 79 - 30 Sep 80 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) JOHN D. MARSHALL, COL, MSC		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Letterman Army Institute of Research Presidio of San Francisco, California 94129		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, Maryland 21701		12. REPORT DATE 1 Oct 80 13. NUMBER OF PAGES 222
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17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*		

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

During Fiscal Year 1980 progress was attained at the Letterman Army Institute of Research in the following research areas: Basic and applied studies on blood, blood products and blood substitutes; physiology of hemorrhagic shock, pharmocological intervention of shock; the determination of coherent radiation exposure thresholds causing damage to the eye, definition and treatment for laser injuries of the skin and eye; military stress and combat effectiveness; evaluation and toxicology of insect repellents; defense against chemical agents. The progress made in this fiscal year is described in the reports of

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Unclassified

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

the work units presented.

FOREWORD

The research conducted at the Letterman Army Institute of Research, Presidio of San Francisco, California, was accomplished in Fiscal Year 1980 under the following Department of the Army projects:

3A161101A91C - In-House Laboratory Independent Research

3M161102BS02 - Basic Mechanisms of Recovery from Injury

3M162770A802 - Military Preventive Medicine

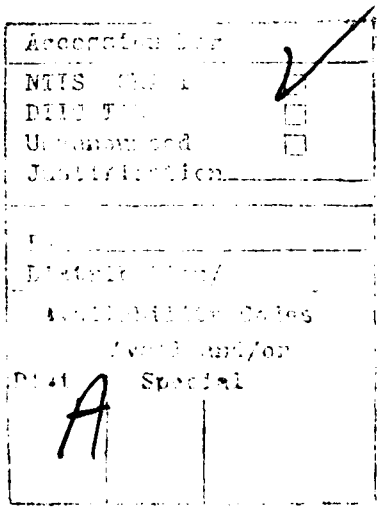
3E162772A813 - Health Effects of Military Lasers

3S162772A814 - Military Trauma and Resuscitation

3E162780A843 - Defense Against Chemical Warfare

Projects are subdivided into work units and studies, as appropriate, to accomplish project objectives.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.



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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6315	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	H. Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	047 APC EL01
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Studies in Behavioral Toxicology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

013400 Psychology; 016200 Stress Physiology; 016800 Toxicology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 12	80 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	2.0	61
	CURRENT 81	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL

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TELEPHONE: (415) 561-3600

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ASSOCIATE INVESTIGATORS

NAME: O'Mara, P.A., MAJ, MS

NAME: Green, M.D., CPT, MS

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Behavioral Toxicology; (U) Toxicology
(U) Neurophysiology; (U) Operant Conditioning; (U) Psychopharmacology

23. TECHNICAL OBJECTIVE. 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Soldiers are routinely exposed to numerous chemical compounds during their performance of military duties. Some of these compounds are known to produce effects which adversely influence the soldier's ability to perform combat-essential activities with maximum efficiency. In general, however, the behavioral effects of chemical exposure are unknown. The objective of this research is to develop and test techniques for detecting and quantifying changes in sensory and motor capabilities attendant to acute or chronic low-level chemical exposure.

24. (U) Animals are exposed to various concentrations of chemical agents. Behavioral and neurophysiological methods are used to assess initial effects on sensory and motor processes and to monitor recovery following chemical exposure. Biochemical and histopathological techniques are used to provide additional information concerning the biological effects of the chemical agents. The effects of exposure to the chemical components of military munitions and combustion by-products will be studied.

25. (U) 7910-8010. An investigation of acute and chronic effects of diisopropylfluorophosphate (DFP) was conducted to assess a behavioral test battery. Biochemical and histopathological techniques were developed to evaluate the effects of various doses of DFP. A microprocessor has been interfaced to control operant conditioning modules. This work unit will be terminated.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 047 — Studies in Behavioral Toxicology

The following investigation has been conducted under this work unit:

STUDY NO. 1 Development of a rapid screening battery

An investigation of acute and chronic effects of diisopropylfluorophosphate (DFP) was conducted to assess a behavioral test battery. Biochemical and histologic techniques were developed to determine the effects of various doses of DFP. An interface and accompanying software have been completed which allow operant conditioning modules to be controlled by a microprocesor for additional behavioral tests.

BODY OF REPORT

WORK UNIT NO.	047	Studies in Behavioral Toxicology
STUDY NO.	1	Development of a rapid screening battery

PROBLEM

Soldiers are routinely exposed to numerous chemical compounds during their performance of military duties. Some of these compounds are known to produce effects which adversely influence the soldier's ability to perform combat-essential activities with maximum efficiency. In general, however, the behavioral effects of chemical exposure are unknown. Proper evaluation of the behavioral test battery requires the use of a chemical which produces known neurotoxic effects. Such a chemical could also be used as a positive control during the investigation of the neurotoxic properties of other compounds.

RESULTS AND DISCUSSION OF RESULTS

A group of albino rats was administered a mixture of diisopropylfluorophosphate (DFP) and peanut oil subcutaneously once a week for 7 weeks. Based on pilot work, a dose range of up to 1.0 mg/kg was used. Twenty-four hours after each injection, a behavioral test battery, including rotating rod, spontaneous alternation, and rapid avoidance, was administered to the subjects. Dosing and limited testing were continued for 8 additional weeks to assess the chronic effects. A second group of rats received weekly testing and doses of DFP up to 2.0 mg/kg for three weeks. To assess possible nerve degeneration, nerves were excised, fixed in osmium, imbedded in Epon, and stained with toluidine blue.

According to the analysis of data to date, the behavioral test battery did not differentiate between the various doses used in these studies. No evidence of chronic behavioral effects was observed. Histopathology with use of a light microscopy technique did not reveal any nerve degeneration.

A digital logic interface was constructed to provide microprocessor control of eight operant conditioning modules. Software for operant conditioning has been completed. This equipment will be used for the evaluation of subtle behavioral effects.

CONCLUSIONS

The behavioral test battery did not differentiate between the levels of DFP used in this experiment. The general absence of effects implies that larger doses are needed to evaluate the test battery appropriately. Pilot work using greater effective doses suggests that at least some of the tests in the battery were sensitive to higher doses of DFP.

RECOMMENDATIONS

The behavioral test battery should be evaluated with the use of other neurotoxic compounds. Operant conditioning techniques should be used to assess changes in sensory processes. Electrophysiological techniques should be used in combination with behavioral methods to evaluate central nervous system and neuromuscular activity.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OE 6316	2. DATE OF SUMMARY* 80 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 79 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8a. DISS'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	CA	203 APC TL08
b. [redacted]	61101A	3A161101A91C	00	049
c. [redacted]	STOG	80-7.2:2		

11. TITLE (Precede with Security Classification Code)* (U) Toxicological Screening of Potentially Hazardous Substances Using Drosophila Melanogaster

12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 002600 Biology; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79-01	81 03	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	2.0	58
	CURRENT 81	4.8	165

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
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NAME: Rutledge, L.C., DAC

21. GENERAL USE
Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Toxicology; (U) Mutagenicity; (U) Drosophila melanogaster; (U) Sex-linked recessive lethal test

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To establish an in-house capability for the toxicological screening of potentially hazardous substances using the Drosophila melanogaster sex-linked recessive lethal (SLRL) test.

24. (U) A D. melanogaster insectary capable of supporting a SLRL testing program has been established and personnel trained in rearing and testing procedures. Exposure methodology will be developed, computer programs for labeling of test insects, data storage, and analysis will be developed, and standard operating procedures (SOPs) will be written to insure compliance with the Good Laboratory Practices (GLP) Regulations. Pilot studies and testing of experimental compounds will be initiated as soon as possible.

25. (U) 7910-8009. Exposure methodology for feeding adult flies water soluble materials has been developed and incorporated into the test system. Equipment required for injection of non-water soluble materials has been ordered. A computer program for labeling test insect vials and experimental groups is currently in use and preparation of data storage and analysis programs is in progress. Nineteen SOPs have been completed to comply with GLP requirements. Pilot studies using a known mutagen to insure proper functioning of the SLRL test system, have been completed. Testing of a water soluble material, 2-ethyl-1,4-benzoquinone, is 50% completed. A solution to a major problem, microbial contamination of the rearing medium, is actively being sought.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 049 — Toxicological Screening of Potentially Hazardous Substances using *Drosophila melanogaster*

The following investigation has been conducted under this work unit:

STUDY NO. 1 Establishing an in-house capability for the toxicological screening of potentially hazardous substances using the *Drosophila melanogaster* sex-linked recessive lethal test

The Armed Forces are often confronted with unique toxicology problems associated with the varied tasks required for mission completion. While many problems facing the military are unique to its environment, Federal requirements must still be met concerning human and environmental exposure to potentially hazardous substances. The Department of Defense does not possess in-house capability for the volume and diversity of compounds that must undergo toxicological testing to meet Federal legal requirements. Establishing an in-house capability for the toxicological screening of potentially hazardous substances by using the *Drosophila melanogaster* sex-linked recessive lethal (SLRL) test is part of the LAIR toxicology program designed to help meet these requirements. Major problems with medium consistency and mold contamination have been resolved. Computer programs and SOPs have been written to insure compliance with the Food and Drug Administration Good Laboratory Practices Regulations. Treatment procedures required for non-water-soluble test compounds are being developed. Laboratory testing of 2-ethyl-1,4-benzoquinone has been completed and the data analysis methodology is being developed.

BODY OF REPORT

WORK UNIT NO.	049	Toxicological Screening of Potentially Hazardous Substances using *Drosophila melanogaster*
STUDY NO.	1	Establishing an in-house capability for the toxicological screening of potentially hazardous substances using the *Drosophila melanogaster* sex-linked recessive lethal (SLRL) test

PROBLEM

Regulations dictate establishment of safety criteria for many new substances proposed for human use or release into the environment. Some of the required tests must be performed before any human contact will be allowed. Other required tests, because they are relatively rapid and inexpensive, are performed as soon as possible to detect unacceptable substances for removal from consideration. The *Drosophila* SLRL mutagenicity test is an example of the latter type of test. After it is established, it will be a potent tool for detecting substances that cause genetic disorders. However, it is not a simple procedure to establish, and considerable training is required before one can consistently interpret results accurately. As a result, there are few laboratories capable of performing the test. This work unit was initiated to determine the feasibility of establishing and maintaining an in-house capability for performing *Drosophila melanogaster* SLRL tests to support Army requirements for toxicological testing.

RESULTS AND DISCUSSION OF RESULTS

Two major problems in the production of *Drosophila* medium were identified and rectified. The agar used in the medium was the source of problems with medium consistency. Increasing the agar concentration and replacing our supply resulted in uniform medium. Mold contamination resulted in a loss of approximately 25% of our usable vials. Potential sources of contamination were identified and swabs/samples were tested for microbial activity by using Tripticase soy plates. The molasses used was identified as the probable source of the contamination and was replaced. Stringent sanitary procedures have been incorporated into SOPs. Two mold inhibitors, propionic acid and methyl p-hydroxybenzoate, are routinely used to reduce the possibility of mold/mite infestations, common problems in *Drosophila* insectaries.

Twenty-four SOPs, covering different phases of the SLRL mutagenicity test, have been written and approved by the LAIR Quality Assurance

Officer. Additional SOPs are being written and approved SOPs are being modified as needed to insure compliance with the Food and Drug Administration Good Laboratory Practice Regulations.

Computer programs have been written and are in use for the generation of Treatment Labels, Brood Cards, and Run Cards. These allow each treated insect, each brood, and each run to receive an unique computer-generated number. This results in a significant saving in time over hand labeling (approximately 95%) and a reduction in the possibility of making duplicate or incorrect identification labels.

An Environmental Mutagen Society committee has recently proposed new statistical criteria in determining significance of test results when using the SLRL system. These criteria will be examined and incorporated into the existing SOP and program if approved by the Chief, Information Sciences.

Establishing an injection capability to allow testing of non-water-soluble materials will require modification of equipment on hand and the purchase of a micro-needle puller. Pilot studies have been initiated to determine the feasibility of using a liposome microencapsulation procedure for feeding of materials insoluble in aqueous solutions.

The laboratory phase of the SLRL testing of 2-ethyl-1,4-benzoquinone (EBQ) has been completed. This involved the examination of approximately 25,000 x-chromosomes from male insects exposed to positive and negative control and test compounds. The program for determining statistical significance is being revised; however, preliminary examination of the data indicated that EBQ is not mutagenic when using a 72-hour 1-mM-exposure dosage. The testing of 2-methyl-1,4-benzoquinone has been initiated.

Solubility tests were conducted on N-octyl-glutarimide, a candidate arthropod repellent. This material, which is not water soluble, will require testing with the injection technique or liposome encapsulation. Both exposure methods are currently being developed.

CONCLUSIONS

An in-house capability has been established for the mutagenicity testing of water-soluble compounds using the *Drosophila melanogaster* sex-linked recessive lethal assay. Tests are conducted under the Food and Drug Administration Good Laboratory Practices Regulations.

RECOMMENDATIONS

This mutagenicity testing capability should be utilized in future toxicological screening by DOD agencies.

Toxicological Screening of Potentially Hazardous Substances using *Drosophila melanogaster* (Cont)

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 0178	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISS'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	050 APC NL04
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)*

(U) Toxicology of Explosives and Explosive By-Products

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

016800 Toxicology; 005900 Environmental Biology; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	1.5	12
FISCAL YEAR CURRENT 81	1.5	125

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

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NAME: LTC,VC; Skala,J.H.,DAC;Hannon, J.P.,DAC

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Military Toxicology; (U) Munitions Chemicals; (U) Carcinogenesis; (U) Teratogenesis; (U) Military Performance

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Under provisions of National Policy Act of 1969, and all other Federal Environmental laws, the U.S. Army Is assigned responsibility for the protection of soldiers during training and combat from chemicals generated by military activities and other activities. Because of the markedly increasing requirements for toxicology testing by industry and government agencies, and a critical national shortage of facilities and trained personnel to address these requirements, the U.S. Army faces and untenable position in discharging its assigned responsibilities. The purpose of this work unit, therefore, is to establish and implement an in-house toxicology program specifically directed to the testing and evaluation of environmental chemical contaminants generated by munitions manuracture and use.

24. (U) Two areas of research will be pursued. The first will be concerned with the test and evaluation of chemicals for mutagenic, carcinogenic, reproductive, or teratogenic effects that may pose a health hazard to humans. The second research area will be concerned with the impact of candidate chemicals on combat-related performance factors and the evaluation of treatment modalities when adverse effects are observed.

25. (U) 7910-8010. Preparation of 2,4-Dinitrotrotoulene (2,4-DNT) was complicated by the compound's insolubility in conventional carrier solvents. This resulted in some initial data being invalid; subsequently, however, a suitable method for accurately dosing animals was developed. Despite somewhat slower than anticipated progress in determining the toxicity of 2,4-DNT, work performed has permitted the evaluation and the modification of SOPs into smooth working documents while being in compliance with EPA and FDA GLP regulations. Studies conducted permitted the evaluation and refinement of the TOXSIS(R) automated data collection system.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	050	Toxicology of Explosives and Explosive By-products

The following investigations have been conducted under this work unit:

STUDY NO. 1 Toxicology of 2,4-dinitrotoluene (2,4-DNT)

EX-1 Determination of LD_{50} for 2,4-DNT in rats and mice

This project was designed to determine the LD_{50} for 2,4-DNT in mice and rats. Difficulties in preparation of 2,4-DNT for oral dosing of animals were encountered. The insolubility of 2,4-DNT in the conventional solvents invalidated the initial study because of variations in the amounts of the compounds delivered to the test animals. A suitable method for accurately dosing animals was subsequently developed by heating 2,4-DNT in Tween 80. The Institute's reorganization resulted in changes of the personnel in key positions. Despite the somewhat slower than anticipated progress in determining the toxicity of 2,4-DNT, the work performed has permitted the evaluation and the modification of SOPs into smooth working documents which are in compliance with the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) Good Laboratory Practice Regulations (GLP).

BODY OF REPORT

PROJECT NO. 3A61101A91C	In-House Laboratory Independent Research
WORK UNIT NO. 050	Toxicology of Explosives and Explosive By-Products
STUDY NO. 1	Toxicology of 2,4-dinitrotoluene (2,4-DNT)
EX-1	Determination of LD_{50} for 2,4-DNT in rats and mice

PROBLEM

The U.S. Army Medical Research and Development Command has the responsibility for evaluating the potential health hazards of all military high explosives and explosive by-products. Exposure to such hazards may occur among workers employed in munitions plants or in the civilian populations as a result of environmental contamination associated with munitions manufacture and assembly. Areas of concern at the present time are the toxicologic effects of 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitrohexahydro-1,3,5 triazine (RDX) and their by-products. These chemicals are discharged into the environment without significant treatment in waste waters resulting from the loading of shells with TNT and RDX mixtures. The waste waters are referred to as LAP (load, assemble, and pack) water which contains a 1.6:1 blend of TNT and RDX, and condensate water which contains a blend of some 30 compounds produced by solar irradiation of TNT/RDX mixtures.

This project is concerned with the acute, subacute, and subchronic toxicology of 2,4-dinitrotoluene (2,4-DNT), a major component (≈ 43% relative concentration) of condensate water. Prior studies by different organizations have addressed this subject, but the results have been inadequate to satisfy requirements for assessment of long-term human health hazards or the establishment of comprehensive environmental standards for this compound. Thus, there is a need for verification of earlier findings. An LD_{50} study, using mice and rats, and subsequent 14-day subacute and 90-day chronic studies will be conducted.

RESULTS AND DISCUSSION OF RESULTS

Considerable difficulty was encountered in finding a suitable carrier solution for 2,4-DNT. Because of its insolubility in H_2O, corn oil was used as a suspending medium. Corn oil suspension of 2,4-DNT was abandoned because the compound formed larger crystals and did not appear to remain in suspension. After extensive experimentation, it was determined by heating 2,4-DNT in Tween 80 to ≈ 65 C it would dissolve.

Then by cooling the solution to 37 C it could be successfully administered by oral dosage. SOPs and protocol formats suitable for GLP compliance were developed.

During this period, the Institute was reorganized, which resulted in changing the Study Director, The Principal Investigator, and many of the technical staff members. The automated data collection equipment and software developed in-house for this and other studies have worked well and saved considerable manpower. However, the TOXSYS equipment and hardware have taken a considerably longer period to become operational than had been anticipated. Efforts to make the system operational are on-going and some progress is being made.

The approximate lethal-dose-range-finding study in rats has been completed. The LD_{50} for both mice and rats is scheduled for completion in early 1981.

CONCLUSIONS

None

RECOMMENDATIONS

Efforts to bring the TOXSYS system to full functional capacity on this study should continue. Continued efforts to utilize TOXSYS on these studies should also be pursued with the hope that TOXSYS will eventually save considerable manpower.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OG 2345	80 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	H. TERMINATION	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C		052 APC 504R
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)* (U) Development of Laboratory Capability for Evaluating Test Formulations as Repellents for Asian Terrestrial Leeches

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
002600 Biology; 005900 Environmental Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	81 06	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	1.1	40
FISCAL YEAR CURRENT 81	0.0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME:* Letterman Army Institute of Research
ADDRESS:* Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
NAME:* Letterman Army Institute of Research
Division of Cutaneous Hazards
ADDRESS:* Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Wilson, Henry R., Ph.D., DAC
TELEPHONE: (415) 561 5485
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Eisenberg, George H.G., Jr., MAJ, MSC POC: DA
NAME:

21. GENERAL USE
Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Terrestrial Leeches; (U) *Haemadipsa*; (U) Leech Repellents; (U) Leech Propagation; (U) Repellent Testing

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) To develop the methodology and maintenance capability required for laboratory evaluation of candidate repellents and repellent formulations for efficacy in repelling Asian terrestrial leeches; to develop procedures for propagation of the leeches in sufficient numbers to support the testing program without further importation of the animals to develop, standardize and implement test procedures required for repellency evaluations.

24. (U) Asian terrestrial leeches (genus: *Haemadipsida*) will be collected and shipped through cooperation of DOD medical research laboratories operating in the endemic areas. Natural habitats will be characterized by climatologic data and physical description of the collection site. Investigations will be directed first toward maintenance of natural vigor and then toward propagation to achieve a self-sustaining colony. Optimization environmental factors and feeding schedules will be emphasized. Substitution of membrane feeding for animals will be explored. Repellency test procedures will be developed which use positive physiologic stimuli as encountered in field conditions.

25. (U) 79 10 - 80 06. Terminated. The principal investigator has resigned and availability of another competent investigator in the near future is unlikely. Before termination, a detailed research protocol had been written, evaluated and approved, cages had been constructed for the leeches, appropriate importation permits had been secured from the Department of Agriculture, and arrangements had been made with the CDR, USAMRU (Malaysia) for obtaining the animals and sending them to LAIR.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	052	Development of Laboratory Capability for Evaluating Test Formulations as Repellents for Asian Terrestrial Leeches

Attacks by Asian terrestrial leeches during the Vietnam war assumed an importance disproportionate to their actual significance as health hazards or pests, primarily because of their adverse effects on morale. A water-and-sweat-impervious leech repellent should be available for issue to troops operating in areas of leech infestation. A staff zoologist who was interested in the problem initiated this project to establish a colony of Asian land leeches and develop suitable methods for assessing the efficacy and water resistance of candidate repellents or formulations. A research protocol outlining the intended approach was prepared; the Commander of the U.S. Army Medical Research Unit, Malaysia, agreed to collect and ship sufficient specimens to start maintenance and breeding studies; and appropriate import permits were secured from the Department of Agriculture. At this point, however, the investigator accepted an offer for a position in private industry and left government service. The protocol has been retained and Institute Reports have been drafted that will insure retention of the knowledge and reference file obtained in preparation for the project so that it can be reactivated when an appropriate investigator becomes available.

BODY OF REPORT

WORK UNIT NO. 052

Development of Laboratory Capability for Evaluating Test Formulations as Repellents for Asian Terrestrial Leeches

PROBLEM

Attacks by Asian terrestrial leeches during the Vietnam war assumed an importance disproportionate to their actual significance as health hazards or pests. Although the area adjacent to the bite often became infected secondarily with bacteria, the primary importance of leech infestations was their adverse effects on morale. Not only were soldiers disgusted upon finding engorged leeches clinging to their bodies, many soldiers were considerably apprehensive because of exaggerated reports of attacks involving invasion of and engorgement within the penile urethra. Diethyl toluamide, the principal insect repellent for troop issue, was effective in repelling the leeches, but efficacy lasted no longer than 30 minutes on individuals who were sweating profusely or were exposed to water. A lanolin-based repellent lasted longer in field tests performed at that time but was never adopted officially and distributed through supply channels. The importance of the morale factor associated with leech attacks in Vietnam suggests that a water and sweat impervious leech repellent should be available for issue to troops operating in areas of leech infestation. If a colony of Asian land leeches could be established, development of a water-resistant leech repellent could easily be incorporated into the current arthropod repellent program conducted by the Division of Cutaneous Hazards. A staff zoologist was interested in the problem and initiated this project in an attempt to establish a colony of Asian land leeches, genus *Haemadipsa*, at LAIR and, if successful, to develop suitable methodology for assessing the efficacy and water resistance of candidate repellents or formulations.

RESULTS AND DISCUSSION OF RESULTS

A research protocol outlining the intended approach was prepared; the Commander of the U.S. Army Medical Research Unit, Malaysia, agreed to collect and ship sufficient specimens to start maintenance and breeding studies; and appropriate import permits were secured from the Department of Agriculture. At this point, however, the investigator accepted an offer for a position in private industry and left government service, forcing termination of the project. The protocol has been retained and Institute Reports have been drafted that will insure retention of the knowledge and reference file obtained in preparation for the project.

CONCLUSIONS

None

Development of Laboratory Capability for Evaluating Test Formulations as Repellents for Asian Terrestrial Leeches

RECOMMENDATIONS

This investigation should be resumed when a suitable investigator becomes available.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 3370	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 01 25	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	053 APC LL01
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Immediate Care of the Combat Wounded

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012900 Physiology; 00800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 01	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	0.1	01
	CURRENT 81	0.1	02

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME: Dixon, R.S. MAJ, VC

NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Hemostasis; (U) Abdominal Cavity; (U) Alginate; (U) Expermiental Animal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) If a liquid material could be infused into the belly to fill all of the dead space, and this liquid then could change its state to a gel, hemostasis would occur by virtue of the blood not having any place to flow. This material would have to be a thin liquid initially, and then be able to change its state to a gel very quickly without the generation of heat. In addition, the gel would have to be able to be placed into solution or be able to be "peeled off" the viscera when definitive treatment became possible at a hospital center. The purpose of this work unit is to find the best material to test the preceding theory, and to see what the physiological effect would be of filling the abdominal cavity of an experimental animal with a gel. Also to see if filling the belly with a gel will provide short-term hemostasis in an animal system.

24. (U) For the pilot study, alginate, the irreversible hydrocolloid used to prepare dental impressions, will be tested initially. Various concentrations of the alginate powder will be mixed with saline and tested for firmness, time for setting, reaction in the presence of whole blood, etc. Other alginate-like compounds will be used as they become available from the manufacturer.

25. (U) 8001-8010. The hydrocolloid, alginate, was used to prepare a different solution. Alginate, 1:6 in normal saline, produced a firm heavy gel. Combining alginate and Triton X-100, a surface tension agent, produced a foamy gel. when Alginate-Triton X was bubbled through the solution, a spongy light gel was provided. This gel was then tested in laboratory rats and did fill the abdomen. Toxicity of the triton and need for a better delivery systems were problems.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 053 — Immediate Care of the Combat Wounded

Various concentrations of alginate, the hydrocolloid used for dental impressions, were tested. A 1:6 solution of alginate in normal saline produced a firm but heavy gel within two minutes. The same solution, when mixed with a surface tension agent, 1% Titron X-100, and stirred in a blender, produced a foamy but still heavy mass. If the alginate-Triton X-100 mixture was prepared with N_2 gas bubbled through the liquid as it gelled, a light, foamy mass was produced. In our preliminary studies in rats, we found that this last preparative process with the alginate-Triton X-100 mixture could fill the abdomen fairly well. If studies along this line are continued, we are going to need a delivery system which is capable of coating the entire cavity consistently each time the procedure is performed.

BODY OF REPORT

WORK UNIT NO. 053 — Immediate Care of the Combat Wounded

PILOT STUDY — Hemostasis in penetrating wounds of body cavities

PROBLEM

The combat medic on the battlefield is faced with a difficult situation when trying to stabilize the vital signs of a patient with a penetrating wound of the abdominal cavity. Although the medic has the capability of infusing blood replacement solutions to treat shock, he does not have the equipment and facilities to stem the flow of a major blood vessel bleeding into the abdomen. In this situation, all of his blood replacement solution may be, in fact, pouring through the damaged vessel into the abdomen. In future conflicts where air superiority may be lacking, evacuation of casualties may take longer than those of the Vietnam war. Those patients with major bleeding vessels of the abdominal cavity may never live to reach a definitive treatment facility.

Some method of temporary occlusion of major vessel bleeding in body cavities is needed. This method must be adaptable to a combat situation and not require sophisticated equipment.

If a liquid material could be infused into the abdominal cavity to fill all of the dead space, and if this liquid then could change its state to a gel, hemostasis might occur by virtue of the blood not having any place to flow. This material would have to be a thin liquid initially, and then become a gel very quickly without the generation of heat. In addition, medical personnel should be able to dissolve or remove the gel when definitive treatment becomes possible at a hospital center.

RESULTS AND DISCUSSION OF RESULTS

The irreversible hydrocolloid, alginate (Jeltrate, L.D. Caulk Co., Milford, DE), which is used to prepare dental impressions, was selected as the initial test material in this pilot study. Using normal saline solution as the diluent, we prepared varying concentrations of alginate (1:1-1:10). A 1:6 concentration of alginate powder in saline seemed to be most suitable. One excellent property of the material was its lack of heat production as it changed from a liquid to a solid state. It produced a firm rubbery gel within 2 minutes of mixing. When this material was instilled into the abdomen of two normal anesthetized rats, it filled the abdomen fairly well. It coated the viscera, producing a "mold" of the organs. When the animals were sacrificed, the gel could

be peeled from the viscera easily. The gel, if allowed to stand for 24 hours or more, began to lose water and to shrivel. When the entire mass had gelled in the abdominal cavity, the weight of the solid was noted. To be effective, such a mass would have to be much lighter to keep from compromising the venous circulation. Next, a surface tension agent, 1% isooctyl phenoxy polyethoxy ethanol (Triton X-100, Rohm & Hass Co., Philadelphia, PA), was combined with the saline/alginate. When mixed in a blender, a foamy gel was produced. However, with a similar water content as the first solution, this solution still produced a heavy gel. To lighten the mixture, nitrogen gas was bubbled through the alginate/Triton X-100 as it was stirred in a Waring blender. The result was a spongy light mass with an adequately firm consistency. This material was then infused into the abdomen of rats by using a perforated infant feeding tube to deliver the material. It gelled within 2 minutes. The animals were allowed to recover from anesthesia. All four animals died within 24 hours. On necropsy, the abdomen was partially filled with the gel. Some portions of the cavity did not have alginate present. The material held its texture well and could be peeled from the intestines, leaving a mold of the organs.

CONCLUSIONS

The basic hypothesis of this study needs further investigation. Better solutions and more effective delivery systems are necessary.

RECOMMENDATIONS

Triton X-100 may have itself been toxic to the animals. Solutions, such as dextrans or other materials should be evaluated in future experiments. In addition, a more effective method of delivery of the material while it is in its liquid state should be developed. A propellant-can arrangement would be ideal if the proper solution can be found. Because of other priorities of the principal investigator, this pilot study will not be continued at this time.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OG 3426	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 07 10	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	054 JL01
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procode with Security Classification Code)*
(U) Isolation of Hematopoietic Stem Cells for Long Term Cryopreservation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 07	83 07	DA	C. IN-HOUSE

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	0.7	26
FISCAL YEAR CURRENT 81	1.0	35

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Bolin, Robert B., LTC, MC
NAME: POC:DA

21. GENERAL USE
Foreign Intelligence Not Applicable

22. KEYWORDS (Procode EACH with Security Classification Code) (U) Stem cell failure; (U) Mononuclear cells; (U) Pheresis (U) Radiation syndrome; (U) Cell harvest; (U) Cryopreservation

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procode text of each with Security Classification Code.)

23. (U) The development of radiation injury by a group of soldiers would result in definable morbidity and mortality according to the exposure. Acute radiation syndrome, above level 1 severity, would require intensive medical support. The treatable and main syndrome is hematopoietic where death can be attributed to hematopoietic stem cell failure. The ability to easily isolate these stem cells, store them in the frozen state for indefinite periods and make readily available to combat theater hospitals would drastically affect morbidity, mortality, and anomic states of irradiated soldiers. This work is directed at development of a rapid procedure whereby mononuclear cells can be frozen for long periods.

24. (U) Mononuclear cells will be harvested, preferably from peripheral blood, by several pheresis procedures. These cells will be evaluated for stem cell functions by feeder layer cultures and/or in vivo splenic implants in rodents. Harvest techniques will be optimized and attempts will be made to further isolate homogeneous stem cell populations by gradient techniques. Bone marrow stem cells will also be isolated. Freezing protocols will be examined for isolated stem cells. The final product(s) will be tested in aplastic dogs.

25. (U)8007-8009. Pheresis procedures have been developed for dogs using a Model 30 Haemonetics machine. Dogs tolerate this procedure without morbidity. Harvest of mononuclear cells (MNC) was studied to determine best yields. The best results were obtained using hetastarch (6%) for 8-10 passes going 2 min into the red cell layer ($x=6.9 \times 10^6$ MNC/ml, max 8.9×10^6 MNC/ml). Cohorts of MNC were separated with isopykinic gradients. A cohort of light density cells, comprising 2% of the total MNC, was isolated. These cells were morphologically different from monocytes or lymphocytes and may be circulating stem cells.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	054	Isolation of Hematopoietic Stem Cells for Long Term Cryopreservation

The following investigation has been conducted under this work unit:

STUDY NO. 1 Pilot study

The development of radiation injury results in definable morbidity and mortality according to the degree of exposure. Acute radiation syndromes may require intensive prolonged medical support. The major treatable syndrome is due to bone marrow failure attributable to various degrees of injury to the hematopoietic stem cells (HSC). The ability to harvest, store, and ingraft HSC would drastically affect morbidity, mortality and psychological states of irradiated soldiers. Attempts to harvest and store HSC conveniently are being investigated. Dogs are used to establish feasibility and techniques that can be applied to humans. HSC from bone marrow and circulating blood are being evaluated. Current studies in dogs show that HSC rich mononuclear cells can be density separated from circulating blood. Investigation into the characteristics of peripheral blood versus bone marrow HSC is now underway. Pursuant to this, primate studies are also being planned to corroborate findings in dogs.

BODY OF REPORT

WORK UNIT NO. 054 Isolation of Hematopoietic Stem Cells for Long Term Cryopreservation

STUDY NO. 1 Pilot study

PROBLEM

The development of a way to store and engraft hematopoeietic stem cells (HSC) should have significant impact on military medicine as well as a psychological impact for involvement in military conflicts with potential nuclear warfare.

The development of radiation injury by a group of soldiers would result, according to the radiation exposure, in a definable morbidity and mortality. The resultant acute radiation syndrome beyond level I severity would require medical support of the majority of those exposed (>200R whole body radiation). Individuals with higher exposure rates (>600R) will require medical attention immediately after exposure; whereas, those with intermediate exposures will recover from the acute prodrome, then develop. after a latent period of 1 to 3 weeks, a severe illness with hematopoietic failure in over half those exposed (level II and level III clinical stages). Although half those receiving level II doses will survive, the chance of survival is greatest in those receiving medical palliation. The main syndrome in levels II and III is the hematopoietic one and death can be attributed to hematopoietic stem cell failure. The $LD_{50/60}$ is estimated between 300 and 500 rads (50% deaths within 60 days) which characterizes the protracted nature of the syndrome as compared to the central nervous system syndrome ($LD_{50/2}$) or the gastro-intestinal syndrome ($LD_{50/8}$). Modern hemotherapy with red cells, platelets, and white blood cell transfusions along with isolation and antibiotics could support victims with the hematopoietic syndrome and could significantly alter current LD_{50} estimates. However, such intensive support is beyond current combat zone capabilities and would be logistically difficult even in CONUS hospitals presently offering specialized hemotherapy. If several victims required simultaneous treatment, support would be impossible in military and most civilian hospitals.

Hematopoietic stem cell transfusions would offer two distinct advantages:

1 HSC transfusions could be given early in the disease. Engraftment would then limit the clinical course to a period of days rather than weeks. This would result in earlier return to duty of survivors, as well as increased number of potential survivors.

2 Conceivably, HSC transfusions given the the prodromal syndrome could eliminate the main phase (hematopoietic syndrome) thereby minimizing medical support requirements.

The psychological importance of this type of medical support is great. The agony of prolonged illness with a high probability of death has a great psychosocial impact on those associated with such experiences. The ability to treat and shorten the time frame of the illness will lessen anomic behavior of associates who are otherwise well-bodied and capable. Hematopoietic stem cell transfusion could also be effective treatment for aplastic anemia due to toxic chemical exposure.

HSC transfusions require the development of technology to harvest and store HSC for future use. Bone marrow harvest and freezing for transplantation is one developing technology addressing the goals of HSC transfusion but is impractical for large scale military needs. The ability to harvest and isolate HSC from blood could provide the logistical technology for the military if harvest of a therapeutic dose can be obtained from a single donor.

RESULTS AND DISCUSSION OF RESULTS

The dog is used as the animal model. Mononuclear cells (MNC) were procured by pheresis procedures from the model. Currently, the pheresis is being performed with Model 30 Haemonetics batch process equipment. Dogs (N=4, replicates of 4) tolerate the procedure but become anemic after 2 to 3 separate runs and thus require iron supplementation (iron dextran) after each run. Ten batch passes can be done during 1 run with volume yields less than 400 ml. MNC harvest has been optimal using hetastarch (6% w/v) added to the anticoagulant (sodium citrate) and going 75 seconds into the red cell layer ($\bar{x}$=6.9 x 10^6 MNC/ml). MNC cohorts have been further isolated and defined by isopyknic gradients. Morphologically distinct cells can be seen according to cell density; less dense cells are large with large cytoplasmic/nuclear ratios whereas heavier cells are small scant cytoplasm cells. The least dense cells (<1.071 gm/ml) are morphologically heterogeneous and composed of 60% monocytoid cells and 40% cells with a nonconvoluted nucleus that has lacy chromatin and may contain up to two nucleoli. The least dense cohort comprises 2% of the total MNC harvested from peripheral blood. Preliminary studies (N=3 dogs) at 10 days show that this least dense fraction has the only Colony Forming Units-culture (CFU-c) activity of the five cell densities studied (1.071 to 1.093 gm/ml). It appears that peripheral HSC are present in peripheral blood of the dog. They can be identified and separated by density gradients.

CONCLUSIONS

In dogs, HSC can be identified in circulating blood by available techniques. These HSC (myeloid HSC) can be isolated by isopyknic gradients without cell damage.

Isolation of Hematopoietic Stem Cells for Long Term Cryopreservation

RECOMMENDATIONS

Further investigations should be done in harvesting technology before progressing into engrafting. Specifically, isopyknic gradients should be developed to isolate cells (gradients from 1.062 through 1.079 gm/ml) further with CFU-c activity. Also, harvesting of HSC from primates must begin immediately to see if the findings in dogs can be confirmed. Colony Forming Units-erythroid (CFU-e) as well as Colony Forming Units-spleen (CFU-s) should be evaluated in the primate model. Optimization of HSC can then be achieved by zonal isokinetic and/or isopykinic gradients.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OG 2880	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	H. Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	055 APC EL02
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) The Role of Superoxide in the Posterior Segment of the Rabbit Eye

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 04		DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	0.5	26
FISCAL YEAR CURRENT 81	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE

Foreign Intelligence Not Applicable

ASSOCIATE INVESTIGATORS

NAME: Belkin, M., LTC, MC, IDF

NAME: POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Eye Damage; (U) Superoxide; (U) Vitreous Bands; (U) Intraocular Trauma

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) On the battlefield, frequent medical complications of eye injury from metallic and non-metallic fragments which penetrate the eye may include vitreal hemorrhage and vitreal band formation. These bands interfere with vision and ultimately may produce retinal detachment. The object of this study is to determine whether vitreous band formation can be alleviated with superoxide inhibitors.

24. (U) Double perforating injuries of a type known to produce vitreous bands in rabbits will be done bilaterally. One eye will receive an injection of a superoxide inhibitor and the other eye used as a control. The eyes will be followed to determine whether the superoxide inhibitor was effective in reducing vitreous band formation.

25. (U) 8004-8009. Comparison of denatured superoxide dismutase with active superoxide dismutase resulted in more band formation with the active enzyme. An RFQ was prepared for extramural support of this study. Final approval has not been received yet. This work unit has been incorporated into Agency Accession Number DA OE 6103.

*Available to contractors upon originator's approval.

DD, FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 055 — The Role of Superoxide in the Posterior Segment of the Rabbit Eye

The following investigation has been conducted under this work unit:

STUDY NO. 1 The role of superoxide in the posterior segment of the rabbit eye model

EX-3 The effect of superoxide inhibitors in preventing vitreous band formation after double perforating wounds to the posterior segment of the rabbit eye model

In an attempt to find a way of preventing vitreous band formation after perforating injury of the posterior segment of the eye, intravitreal injections of superoxide dismutase and triamcinolone were given individually in doubly perforated eyes of rabbits. Two observers, independently and unaware of which injections had been given, evaluated the extent of band formation two weeks after injury. Their observations indicated that eyes receiving superoxide dismutase developed more band formation than control eyes. In another series of animals, the band formation following injection of denatured superoxide dismutase did not differ from the band formation which developed after injections with active superoxide dismutase. Triamcinolone injections did not significantly reduce band formation compared with control eyes. Further biochemical studies need to be done to determine the reason superoxide dismutase promoted band formation instead of reducing it in this animal model.

BODY OF REPORT

WORK UNIT NO.	055	The Role of Superoxide in the Posterior Segment of the Rabbit Eye
STUDY NO.	1	The role of superoxide in the posterior segment of the rabbit eye model
	EX-3	The effect of superoxide inhibitors in preventing vitreous band formation after double perforating wounds to the posterior segment of the rabbit eye

PROBLEM

After perforating injury to the posterior segment of the eye which produces a vitreous hemorrhage, vitreous bands frequently develop. These bands may obscure vision and may eventually cause a traction detachment of the retina. Vitrectomy is the accepted method of treatment, but due to surgical complications, it is not entirely satisfactory. Since this is one of the most common types of combat injuries to the eye, a better method of treatment is being sought.

Theoretically, it should be possible to inhibit vitreous band formation by intravitreal injection of a substance that interferes with the pathogenesis of the bands. Superoxide is a highly reactive oxygen radical known to be important in collagen formation.

Superoxide dismutase (SOD) is a superoxide scavenger. The addition of this enzyme should have the effect of reducing collagen formation which composes the vitreous bands. Steroids are known to inhibit fibroblast proliferation, therefore, it is possible that vitreous band formation could be prevented by using a potent long-acting steroid such as triamcinolone.

RESULTS AND DISCUSSION OF RESULTS

Bilateral double perforating injuries were produced in the eyes in a series of rabbits. In a blinded fashion, an injection of either SOD or saline was given intravitreally immediately after injury in some of the animals and 24 hours later in other animals. The eyes of the animals were examined independently with an ophthalmoscope two weeks later by two observers, and the band formation was quantified. Their observations indicated that the eyes receiving the superoxide

dismutase had more band formation than control eyes. To determine whether this was due to enzyme action or to some other biological effect of the SOD, eyes of another series of animals were injected with active SOD versus denatured SOD. No difference in band formation was noted between these eyes. In order to evaluate the significance of these results, we need to know the normal superoxide level in the eyes of the vitreous of the rabbit, the level after injury and hemorrhage, and the level after SOD injection. This will be done after the extramural contract is approved.

After triamcinolone was injected, no significant difference in band formation could be found compared to control eyes.

CONCLUSIONS

Neither superoxide dismutase or triamcinolone inhibit vitreous band formation in the rabbit eye model.

RECOMMENDATIONS

Further biochemical studies are needed to determine whether or not manipulation of the superoxide concentration in the vitreous will have a significant effect on vitreous band formation.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 3427	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 07 01	H. Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	056 APC EL10
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Laser Induced Retinal Edema

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

009600 Masers and Lasers; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 07		DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	0.1	25
FISCAL YEAR CURRENT 81	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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TELEPHONE: (415) 561-3600

20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Weiss, J.F., LTC, MC

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Laser; (U) Eye; (U) Retina; (U) Retinal Damage; (U) Retinal Edema

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective of this study is to establish a method for diagnosing and monitoring the progression of laser induced retinal edema. Such a method is currently unavailable. This method will provide a diagnostic clinical tool as well as means of investigating the efficacy of potential treatment modalities of these lesions.

24. (U) Retinal edema will be produced in monkeys' eyes by Q-switched neodymium laser. The lesions will be diagnosed and their progression monitored by A and B mode ultrasound.

25. (U) 8007-8009. There has been no change in the state of this project since the last summary dated 6 August 1980. This work unit has been incorporated into Agency Accession Number DA OE 6103.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 056 — Laser-Induced Retinal Edema

The following investigation has been designed under this work unit:

STUDY NO. 1 Ultrasonic diagnosis and monitoring of laser-induced retinal edema

Work on this study will be initiated in FY 81.

BODY OF REPORT

WORK UNIT NO.	056	Laser-Induced Retinal Edema
STUDY NO.	1	Ultrasonic diagnosis and monitoring of laser-induced retinal edema

PROBLEM

Laser-induced retinal edema is expected to be one of the most common disabling injuries to soldiers on the battlefield in the future. No objective clinical method currently exists for diagnosing and monitoring ophthalmoscopic lesions of this type. We will attempt to solve this problem by using A and B mode ultrasound.

RESULTS AND DISCUSSION OF RESULTS

None

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 3429	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	057 APC LL02
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) The Effects of Sensory Denervation in the Care and Management of Traumatic Wounds

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology; 003500 Clinical Medicine; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 05	83 07	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	0.1	52
FISCAL YEAR CURRENT 81	0.1	25

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research

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RESPONSIBLE INDIVIDUAL

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TELEPHONE: (415) 561-3600

20. PERFORMING ORGANIZATION

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ADDRESS:* Division of Research Support
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) POC:DA

NAME:* Jennings, P.B., Jr., LTC, VC

TELEPHONE: (415) 561-3876

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Dixon, R.S., MAJ, VC

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Sensory denervation; (U) Resuscitation; (U) Domestic animal; (U) Combat injuries

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To produce an animal model that can be completely deprived of sensation to a selected area of the body; to study the physiological effects of maintaining the pain-free (anesthetic) state for a prolonged period of time. This will involve monitoring procedures and the insertion of chronic indwelling catheters; and to determine the physiological effects of chronic sensory denervation on the healing of experimental wounds.

24. (U) In the experiments, rhizotomy will be used as the surgical procedure. If this does not prove effective, other techniques will be evaluated (cordotomy, continuous epidural anesthesia). For these studies swine will be utilized. The animals will be maintained as long as humanely possible to evaluate the long-term effects of the procedures, but no longer than 60 days post surgery. Response testing will be performed on a regular basis to see if any return (or additional loss) of sensory function occurs.

25. (U) 80 05-80 10. The technique of dorsal (posterior) rhizotomy was developed to produce sensory denervation by severing selected dorsal roots of lumbar spinal nerves on the right side. After use of a few cadavers and non-survival surgical procedures to evolve techniques, the surgery was refined to allow approach to each dorsal root. The procedure can now be performed in less than two hours. Bleeding is not a problem and postoperative recovery is uneventful. All animals walk post op. Roots severed are L_3 through L_6, and various combinations of one or more of these roots. At present 8 pigs are alive and well following surgery.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3A161101A91C In-House Laboratory Independent Research

WORK UNIT NO. 057 The Effects of Sensory Denervation in the Care and Management of Traumatic Wounds

The following investigations have been conducted under this work unit:

STUDY NO. 1 Anatomy of the spinal nerves in the pig

STUDY NO. 2 Development of the surgical technique for dorsal rhizotomy in the pig

STUDY NO. 3 Measurement of sensory evoked potentials in the pig

STUDY NO. 1. Dissection of pig cadavers revealed the cauda equina begins at the caudal portion of the last (6th) vertebra. Durotomy was required to visualize adequately the dorsal and ventral spinal roots.

STUDY NO. 2. The surgical techniques for performing dorsal rhizotomy in the young pig were developed. Trephine holes in the dorsal laminae of lumbar and sacral vertebrae, coupled with durotomy, allowed identification of individual nerve roots. Dorsal roots of L_3-L_6 were severed on one side and animals were evaluated postoperatively.

STUDY NO. 3. Measurement of sensory evoked potentials in the pig was performed by using a Grass stimulator and a Nicolet Med 80 signal average computer. The sciatic and femoral nerves were isolated in intact anesthetized pigs and evoked potentials were recorded along the proximal portion of the isolated nerves at the dorsal roots and along the lumbar and thoracic spine. Recorded potentials from the brain proved to be unworkable in this model and present studies will concentrate on records from spinal cord electrodes.

BODY OF REPORT

WORK UNIT NO. 057 — The Effects of Sensory Denervation in the Care and Management of Traumatic Wounds

STUDY NO. 1 — Anatomy of the spinal nerves of the pig

PROBLEM

The domestic pig has become popular as an animal model of human disease, especially because its cardiovascular physiology simulates man more closely than most other species. Investigators at LAIR are examining the feasibility of producing an animal model for evaluating the long-term effects of traumatic injuries. The animal must simulate the awake combat-injured soldier, and so animals maintained under general anesthesia are unacceptable. The possibility of a pig model in which sensory innervation to a selected area has been removed while leaving the motor function intact has interesting possibilities in this context. The possible methods to achieve this denervation include rhizotomy of the sensory spinal roots, cordotomy of the lateral spinothalamic tracts, and continuous spinal anesthesia.

RESULTS AND DISCUSSION OF RESULTS

The first study in this work unit was a series of cadaver dissections to determine the anatomy of the pig spinal cord, the position of nerve roots, ganglia and tracts, and the best methods of approaching the pig spinal cord with a minimum of trauma, bleeding, and postoperative complications. The literature did not provide much assistance. Few people, apparently (from the American and European literature we found), have paid much attention to the neuroanatomy of the pig spinal cord in the context of acquiring access to the cord and nerve roots. Some old German texts provided basic landmarks, but no one seemed interested in neurosurgery in the pig. We euthanized two young pigs and exposed the lumbar vertebrae. Because the dorsal spine and dorsal laminae are cartilaginous in the young pig, these were removed easily with Rongeurs. From this laminectomy approach, we could not differentiate dorsal spinal roots from ventral spinal roots without removing the dura mater. In addition, it was virtually impossible for the surgeon to separate nerve fibers by gross inspection. Some form of magnification would be needed when this procedure is performed on a living animal. The termination of the spinal cord in the pig was found to be at the L_6-S_1 position. Large venous sinuses were seen in the spinal cord at the 3 and 9 o'clock positions. The location and large size of these sinuses indicated that

the entrance into the spinal canal would have to be as dorsal as possible to preclude extensive hemorrhage during surgery.

CONCLUSIONS

It is possible to gain access to the lumbar and sacral vertebrae of the pig by using a standard dorsal laminectomy or hemilaminectomy approach. The young pig is ideal because of the cartilaginous vertebrae and minimal amount of epidural fat. For proper identification of spinal roots and nerve tracts, the operating microscope will be necessary.

RECOMMENDATIONS

The surgical approach should be developed initially by using live animals sacrificed immediately after surgery and later in animals allowed to survive.

PUBLICATIONS

None

STUDY NO. 2 — Development of the surgical technique for dorsal rhizotomy in the pig

PROBLEM

Rhizotomy, the severing of selected dorsal (sensory) spinal roots, was selected as the initial surgical procedure to provide sensory denervation to the quadriceps and hamstring muscle areas of the pig thigh. Cordotomy, the severing of the lateral spinothalamic tract where pain fibers course, was considered too radical at this time because of the potential side-effects including motor functional loss. The third alternative, continuous epidural anesthesia, was considered only as a last resort because of the need to eliminate any systemic drug interference in the animal model.

RESULTS AND DISCUSSION OF RESULTS

Pigs 1-7. The initial surgical approach utilized in the live pig was a total laminectomy of L_3 and L_4. The dorsal laminae were removed, the dura incised and inspected. Roots on the right side were identified, stimulated with a nerve stimulator, and sensory roots severed. The dura was not closed but overlaid with absorbable gelatin sponges (Gelfoam), and the fascia and skin closed. The procedure was then modified by exposing the right spinal roots by a burr hole made in the right dorsal lamina over the root. To minimize skin and muscle bleeding while approaching the vertebrae, the Bovie electrocautery unit was used for skin, fascia, and muscle.

The Effects of Sensory Denervation in the Care and Management of Traumatic Wounds (Cont)

Pigs 8-17. The surgical procedure was improved and the time for performing the surgery was reduced to less than 90 minutes, depending upon how many dorsal roots were severed. The major modification was the positioning of the burr hole as far dorsally on the vertebrae as possible. This allowed excellent exposure of the cord and roots, and kept the operative exposure site away from the vetebral venous sinuses which, in the pig, can produce massive bleeding if injured inadvertently. After the trephine hole was complete in the dorsal laminae, the ligamentum flavum was incised by using a dural hook and scissors. For this and all subsequent procedures, the operating microscope was used. The epidural fat was teased apart to expose the dura mater. The dura was incised over the roots by using a dural hook and scissors. Individual nerve fibers from the dorsal roots were isolated and elevated. A nerve stimulator was placed on these fibers to insure they were, in fact, sensory. If no motor responses were produced when these fibers were stimulated, they were severed with a scissors. This procedure was repeated for all nerve fibers until all sensory fibers were cut. The dura was not closed, but the trephine opening was packed with Gelfoam. The same procedure was repeated for additional roots. Prior to closure of the operative site, Gelfoam was layered over all the packed trephine holes. A layered fascial closure, using 3-0 polyglycolic acid sutures, was followed by skin closure with non-absorbable sutures.

All animals in this group of 10 walked postoperatively, regardless of whether one or more of roots L_3-L_6 was severed. No appreciable bleeding occurred. Two pigs died from causes unrelated to the procedure (gastric dilation). One pig developed a rectal prolapse, which may or may not have been related to the surgery, and was sacrificed. The other seven pigs did well. Initially, a few showed some proprioceptive difficulties for a few days after the surgery, but soon adjusted their gait to compensate and became entirely normal within two weeks. All pigs in the group were sacrificed in September because they were becoming too large to handle.

The entire surgical procedure was documented on video tape for future reference.

CONCLUSIONS

A surgical procedure was developed to expose the dorsal spinal roots of the lumbar vetebrae in the pig. The procedure was refined to eliminate significant bleeding and to provide adequate exposure in a short period of time. This procedure can be modified to go up or down the spinal cord as required during the subsequent studies in this work unit.

The Effects of Sensory Denervation in the Care and Management of Traumatic Wounds (Cont)

RECOMMENDATIONS

The investigators feel that the surgical procedure can be performed accurately, and the neurophysiological evaluation of the model should receive priority.

PUBLICATIONS

None

STUDY NO. 3 — Measurement of sensory evoked potentials in the pig

PROBLEM

How does one measure pain in an animal? Do animals perceive pain by the same neurophysiologic mechanism that humans perceive it? Can the neurophysiologic correlates of the animal's pain be measured? These questions are difficult to answer but are crucial to these studies in which we are attempting to produce an animal model which will simulate the awake-combat-injured soldier.

The responses of the pig are not amenable to interpretation by the standard diagnostic neurologic examinations. In addition, as pigs grow larger, they become more aggressive and are not suitable for handling unless they are sedated.

One newer diagnostic technique for recording "pain" involved placing recording electrodes on the brain or spinal cord. Recording the electrical potentials which are produced in the sensory circuit (when a small electrical stimulus is applied to a peripheral nerve or to skin and muscle supplied by a particular peripheral nerve with a sensory component) assists us in our evaluation of the response to pain by this animal model, i.e., the pig. The results may not, however, prove beyond the shadow of a doubt aht the is/or is not really feeling pain. Nevertheless, this technique is the most sophisticated method we have at the present time. In these studies, we will interrupt the sensory nerve innervation to a selected area of the body (i.e., one hind leg of the pig), and will try to determine by recording the electrical potentials if the animal perceives "pain" when a noxious stimulus to this denervated area is applied.

RESULTS AND DISCUSSION OF RESULTS

Initial experiments utilized anesthetized pigs which had not had surgery previously. Stimulation to the hind limb was produced by using a Grass instrument stimulator and needle electrodes placed at various points in the skin. Recording needle electrodes were placed in the scalp. The Nicolet Med 80 was used as a signal averager.

Difficulties were encountered in recording from the brain because the strength of the stimulus needed to evoke responses on the electroencephalograph were at such levels to "electrify" the whole cord. For the next experiments, direct stimulation of major nerve trunks was performed. The tibial nerve was isolated medial to the achilles tendon and the sciatic nerve was isolated in two locations, distally beneath the biceps femoris muscle, and proximally at the greater trochanter of the femur. In addition, the dorsal nerve roots over L_5 and L_6 were isolated as well as the large tract at S_1. Electrical stimulus was applied to the tibial nerve and recordings made proximally on the sciatic and along the nerve roots. Evoked responses were recorded. Technical problems of keeping the nerve and electrode "insulated" from the rest of the animal were solved by suspending the isolated segments in a pool of mineral oil.

Subsequent studies will investigate the possibility of recording the potentials using needle electrodes placed in the general vicinity of the lumbar spinal cord. This will obviate the need for surgery and make the technique feasible under short-acting anesthesia. Arrangements have been made to consult with a veterinary clinical neurologist at the School of Veterinary Medicine at Davis, CA. This individual is studying the use of spinal evoked potentials in the diagnosis and treatment of spinal cord disease. His expertise will be helpful in this project.

CONCLUSIONS

Further study is needed to allow recording of sensory (spinal) evoked potentials. The spinal cord will be utilized, not the brain. When responses are satisfactory in the normal pig, mapping of sensory loss following severing of selected roots will be performed. This will enable the surgeons to delineate the area of sensory loss and modify the rhizotomy procedure to attain total desensitization of the desired muscle groups.

RECOMMENDATIONS

The perfection of the spinal evoked potentials as an evaluation of sensory loss should be pursued as quickly as possible. Any further surgical modification to denervate the sensory portion of the hamstring muscle groups should be delayed until the investigators are satisfied with the progress of Study No. 3.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 3431	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	058 APC FL 11
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) An athymic nude mouse-grafted human skin model.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012900 Physiology; 007900 Indust (occupational) medicine; 017100 Weapons effects

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 06	81 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	0.1	01
FISCAL YEAR CURRENT 81	2.0	110

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Reifenrath, William G,. Ph.D., DAC

NAME: Jederberg, Warren W,II,CPT,MSC,POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Skin; (U) Dermal; (U) Cutaneous; (U) Animal Model; (U) Nude Mouse; (U) Skin Permeability; (U) Percutaneous Penetration; (U) Xenograft

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The nude (nu/nu) mouse-grafted human skin model will be established at LAIR and evaluated for its usefulness as a tool for investigating skin permeability, the physiology of dermal penetration, and mechanisms involved in sustaining or preventing injury to skin and in healing injured skin. This model will be particularly useful for investigating problems, like medical defense against chemical warfare agents, which have no civilian counterpart and which entail hazards that preclude use of human volunteers as investigational subjects.

24. (U) A colony of nude mice will be established at LAIR. An investigator with established expertise in developing and using the nude mouse-grafted human skin model will be obtained on a mobility assignment for 9-12 months under the Intergovernmental Personnel Act of 1970 to supervise and participate in the investigation and to teach the LAIR staff how to perform the grafting procedures and successfully maintain and use the experimental animals. The dermal penetration studies will be performed using modifications of procedures that have been used already with man and other laboratory animals.

25. (U) 80 05 - 80 08. The research protocol has been approved, initial purchases of breeding stock have been made, and the nude mouse housing and experimental facilities have been prepared. Also, agreement has been reached with Dr. Gerald Krueger, University of Utah College of Medicine, to come to LAIR in FY 81 on a mobility assignment consisting of one week/month for 9 months and one 3-month period.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 058 — An Athymic Nude Mouse-Grafted Human Skin Model

An athymic nude mouse colony has been started at LAIR and a mobility assignment has been negotiated with Dr. Gerald Krueger, a research dermatologist at the University of Utah College of Medicine. He will come to LAIR and teach our staff how to graft human skin to the animals and successfully maintain them, and use them for research investigations. The human skin grafted on athymic nude mice will provide a model with great potential for use in place of humans. This would, therefore, allow us to study percutaneous penetration and reaction of chemicals which are toxic or which might be harmful to people.

BODY OF REPORT

WORK UNIT NO. 058

An Athymic Nude Mouse-Grafted Human Skin Model

PROBLEM

The Division of Cutaneous Hazards conducts basic and applied research to provide solutions to problems connected with injury to or through the soldier's skin. Presently, the most active and militarily important research programs in this division are: (a) the physiology of dermal penetration, (b) development of skin decontamination technology, (c) toxicologic assessment of skin decontamination materials and (d) development of topical repellents against militarily important arthropods.

The first two programs involve testing of toxic chemicals and radionuclides at dosage levels that are too hazardous for intentional human exposure; the third program is for the specific purpose of determining if human exposure is permissible; and the fourth program involves non-human testing for efficacy followed by toxicologic assessment to insure safety before human exposure. Thus, all require non-human models to estimate efficacy or safety in humans. Each of the in vitro and animal models that is being used to fulfill these requirements is adequate, to a lesser or a greater extent. However, none of the models is adequate for all requirements, and none of the models is adequate for answering many mechanistic questions that arise (e.g., mechanisms of certain skin diseases or of injury by chemicals like vesicants). A need exists for a means to study live human skin that is active and functioning in a normal or relatively normal manner without using human subjects. Athymic nude mice accept human skin grafts. This type of mouse has been shown to be a useful tool for studying skin and skin diseases. However, this is a relatively new and exacting technology. The purpose of this project is to establish the capability at LAIR for grafting human skin to nude mice and to evaluate the potential of the nude mouse human skin model for studying dermal penetration.

RESULTS AND DISCUSSION OF RESULTS

Appropriate facilities for housing and maintaining the animals have been prepared and a breeding program has been started to produce an average of 60 homozygous (nu/nu) offspring per month. A mobility assignment (Title IV, Inter-governmental Personnel Act of 1970) has been negotiated with Dr. Gerald Krueger, a research dermatologist at the University of Utah College of Medicine, who has extensive experience with development of this model. He will come to LAIR to teach us the techniques for making and maintaining the grafts on the animals and to collaborate in the dermal penetration studies. If these studies are successful and permeability values obtained with the model are not significantly different from those observed with human volunteers we will begin using the model in the basic and applied research programs.

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE6114	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	H.Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS02	00	061 APC 506E
b. [redacted]	62772A	3M162772A812	00	005
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)*

(U) Disease Mechanisms at the Cellular Level

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology; 010100 Microbiology; 002300 Biochemistry; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	80 09	DA	A. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	0.4	34
FISCAL YEAR CURRENT 81	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research

ADDRESS:* Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME: Marshall, J.D., Jr., COL, MC

TELEPHONE: (415) 561-3600

20. PERFORMING ORGANIZATION

NAME:* Letterman Army Institute of Research

ADDRESS:* Division of Research Support
Pathology Services Group
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)

NAME:* Mellick, P.W., LTC, VC

TELEPHONE: (415) 561-3855

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: POC:DA

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Histochemistry; (U) Electron Microscopy; (U) Diagnosis; (U) Infection; (U) Laboratory Animal; (U) Metabolic Disease

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Prevention and control of disease depends on complete understanding of abnormal processes involved, from initial cellular injury to repair. Providing pathology support to LAIR's varied research programs requires continued development of highly specialized techniques and accurate diagnosis of spontaneous disease in experimental animals. This project will develop improved methods to support LAIR investigators. It will provide information on cellular response to injury and differentiate naturally-occuring diseases from experimentally-induced lesion.

24. Improved techniques for pathology support will be developed and tested. Histopathology, histochemistry, electron microscopy, and quantitative analytical methods will be used. Accurate diagnoses of diseases in LAIR's laboratory management and accurate interpretation of experimental results.

25. (U) 8008-8010. Histological methods for obtaining high-quality histologic sections of the eye were developed. By embedding ocular tissues in glycol methocrylate and adapting routine histologic stains to this process, sections, 1µm in thickness, were prepared and evaluated. A study was conducted to determine the nature and cause of skeletal muscle lesions observed in colony guinea pigs. Animals from a study that was being terminated were used. Results indicate vitamin E and/or selenium deficiency caused myopathy observed. This work unit is being terminated due to realignment of funding and mission priorities.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3M161102BS02 — Basic Mechanisms of Recovery from Injury

WORK UNIT NO. 061 — Disease Mechanisms at the Cellular Level

The following investigations have been conducted under this work unit:

STUDY NO. 1 — Improved histological, histochemical and ultrastructural techniques of experimentally induced and naturally occurring diseases of laboratory animals

STUDY NO. 2 — Characterization of the nature and cause of striated muscle lesions observed in colony guinea pigs

UNNUMBERED — Reported under work units of other LAIR investigators

Prevention and control of disease, documentation, and accurate interpretation of experimental results in laboratory animals depend upon a complete understanding of abnormal processes involved, from initial cell injury to repair. Providing pathology support to LAIR's varied research program requires continued development and modification of highly specialized techniques and accurate diagnosis of spontaneous disease in experimental animals.

STUDY NO. 1. A technique to prepare high quality thin (1 µm) histologic sections of eyes was developed and evaluated. The procedure involves modifying the preparation of commercially available glycol methacrylate as the embedding media and a standard microtome so that complete cross-sections of large eyes can be cut at a thickness of 1 µm. This method yields sections free of the artifacts usually produced by paraffin embedding and greatly improves histologic resolution.

STUDY NO. 2. Using guinea pigs made available from a study being terminated, we studied the nature and cause of severely debilitating striated muscle lesions that had been observed in the LAIR colony. The disease was reproduced by feeding guinea pig diets deficient in selenium and vitamin E. Histologically, the lesions induced experimentally were nearly identical to those that occurred spontaneously in the LAIR colony and in animals used as controls in several research projects. These results emphasize the importance of storage and utilization of guinea pig feeds in a manner that will prevent degradation of vitamin E.

BODY OF REPORT

WORK UNIT NO.	061	Disease Mechanisms at the Cellular Level
STUDY NO.	1	Improved histological, histochemical and ultrastructural techniques of experimentally induced and naturally occurring diseases of laboratory animals

PROBLEM

Pathology support for studies designed to evaluate subtle effects on intraocular structures requires hiqh quality histological preparations of the eye. Routine methods using paraffin embedding result in numerous artifacts. Sections from paraffin-embedded blocks must be cut at a thickness of at least 5 μm. The thickness of these sections decreases microscopic resolution considerably and makes detection and interpretation of subtle changes difficult. Electron microscopic examination permits high resolution; tissue processing procedures induce few artifacts. However, the size and number of the specimens that can be processed and examined by electron microscopy are quite limited and procedures are time-consuming and expensive. There is a need for high quality histologic preparations of complete cross-sections of eyes. The sections produced should be free of processing artifacts and permit detection of subtle changes. In order to be applicable to the large numbers of specimens required by some studies, the technique should be rapid and inexpensive. Glycol methacrylate has been used successfully as an embedding medium for tissues where high quality histologic preparations are required. However, a limiting factor with this technique is the size of the specimen. When large specimens are embedded, the heat produced by polymerization of the embedding medium causes deterioration of the specimen. In addition, the size of glass microtome knives usually used for sectioning of methacrylate-embedded tissue restricts the size of the specimen to 7 mm or less. The purpose of this study was to modify embedding procedures and equipment so that high quality glycol methacrylate embedded sections of complete cross sections of eyes from larger species of experimental animals can be prepared.

RESULTS AND DISCUSSION OF RESULTS

By altering the relative amounts of the components of commercially available glycol methacrylate embedding material, it was possible to reduce the rate of polymerization so that excessive heat was not produced. This solved the problem of specimen deterioration which had previously limited specimen size. A standard microtome was modified

so that "Ralph" knives (glass knives 38-mm wide) could be mounted. With these knives and large methacrylate embedded sections, it was possible to produce high quality histologic sections of eyes as large as 3 cm in diameter. Sections can be cut at thicknesses of 1-2 μm which greatly increase histologic resolution.

CONCLUSIONS

The embedding medium is compatible with most histologic stains in routine use. The availability of this procedure enhances pathology support for studies requiring histologic evaluation of the eye.

RECOMMENDATIONS

These improvements should be incorporated into the routine procedures available for pathology support to Institute research projects.

PUBLICATIONS

None

STUDY NO. 2 — Characterization of the nature and cause of striated muscle lesions observed in colony guinea pigs

PROBLEM

Severe debilitating lesions in striated muscles of guinea pigs maintained as experimental animals in the LAIR colony have been observed on several occasions (c. 1974-76). Histologic characteristics of these lesions resembled nutritional myopathy caused by vitamin E/selenium deficiency in other species. However, the requirements for selenium and vitamin E in this species have been inadequately studied. Furthermore, some of the animals suffering from this disease had been consuming synthetic diets that had been supplemented with vitamin E. In order to determine whether vitamin E and/or selenium deficiency caused these lesions, a brief experiment was conducted using animals from another study which was being terminated. Animals were divided into the following four diet groups: Group 1 - synthetic diet supplemented with vitamin E and with selenium.; Group 2 - synthetic diet supplemented with vitamin E deficient in selenium; Group 3 - synthetic diet supplemented with selenium deficient in vitamin E; and Group 4 - synthetic diet deficient in both vitamin E and selenium. Animals were maintained until clinical evidence of myopathy was discerned, at which time they were killed and necropsies were performed. Control animals (Group 1) were sacrificed periodically throughout the study for comparison.

Disease Mechanisms at the Cellular Level (Cont)

RESULTS AND DISCUSSION OF RESULTS

All animals in Group 4, deficient in both selenium and vitamin E, had severe degenerative lesions in skeletal muscles. Lesions were present in all of the striated muscles examined including semimembranosis, semitendinosis, intercostal muscles, laryngeal muscles, and tongue. Several animals had degenerative lesions in the myocardium. Lesions similar in nature but less severe were observed in muscles of guinea pigs that consumed diets deficient in vitamin E but supplemented with selenium. No clinical abnormalities or histologic evidence of muscle damage were observed in animals deficient in selenium but supplemented with vitamin E, or the animals supplemented with both selenium and vitamin E.

CONCLUSIONS

Vitamin E deficiency in guinea pigs causes degenerative myopathy. Selenium deficiency alone with vitamin E supplementation does not result in myopathy in this species. Absence of both vitamin E and selenium causes more severe lesions in guinea pig muscle than does vitamin E deficiency alone. Cases of degenerative myopathy observed in LAIR guinea pigs (c. 1974-76) was probably due to vitamin E deficiency. It is likely that the vitamin E in the diets of thesee animals deteriorated during storage even though the vitamin had been added to the commercial product.

RECOMMENDATIONS

Feed for guinea pigs should be stored and utilized in a manner that will prevent deg adation of vitamin E.

This work unit will be terminated at the end of FY 80, due to realignment of mission priorities and funding allocation.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OG 2375	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISS'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	081 APC FL 03
b. [redacted]	61102A	3M161102BS02	00	063
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Prevention and Treatment of Battlefield Infections

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	2.3	53
FISCAL YEAR CURRENT 81	1.4	43

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Jederberg, Warren W. II, CPT, MSC

NAME: Jennings, Paul B., LTC, VC, POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Skin; (U) Cutaneous; (U) Infection; (U) Immunity; (U) Iron; (U) Disease; (U) Models; (U) Battlefield; (U) Casualty; (U) Dermal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) New weapons systems, new options for combat casualty management, and alterations in battlefield evacuation times for casualties may alter the course and the hazards of battlefield infections. Studies are needed to assess the degrees of risk and types of infection likely to occur with different kinds of battlefield injuries or battlefield casualty management techniques and, where appropriate, to develop prophylatic measures to limit incidence and severity of those infections.

24. (U) Functional immune profiles will be used in animal models selected for each study on the basis of compatability of responses with those known to occur in humans. Animal models will be used to assess hazards of infections and efficacy of proposed preventive measures with major traumatic and minor wounds. Risk-benefit assessments will be made considering the type of injury, treatment or intended prophylatic measure.

25. (U) 79 10 - 80 09. Implantation of a percutaneous observation and sampling window in rabbits for studying the dynamics of wound infections did not prove to be feasible. Chemotactic assays were performed in male rats. Zymosan treated sera, bacterial lipopolysaccharide, and filtrate from *E. coli* cultures were used as attractants. No consistant increase in the number of ficoll-hypaque separated peripheral blood mononuclear cells was seen migrating in the presence of any of these substances. Non-specific esterase stains of the mononuclear cell preparation failed to demonstrate esterase activity in any of the cell populations tested. Wright's stains of these same preparations evidenced the presence of lymphocytes and monocytes.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M161102BS02 — Mechanics of Recovery from Injury

WORK UNIT NO. 063 — Prevention and Treatment of Battlefield Infections

The following investigations have been conducted under this work unit:

STUDY NO. 1 Establishment of the methods and baseline data required to conduct a normal host immune profile

EX-1 Establishment of the capability to perform a host immune profile in Sprague-Dawley rats

STUDY NO. 2 Care and management of contaminated and infected wounds in the combat soldier

EX-1 Development of an animal model for study of wound contamination and infection

STUDY NO. 1, EX-1. Histologic specimens were collected from 10 normal rats. Chemotactic assays were performed on mononuclear cell preparations from 25 rats. Zymosan-treated rat sera, Escherichia coli culture filtrate and lipopolysaccharide failed to demonstrate significant chemotactic activity. Smears of the cell preparations demonstrated the presence of 83±7% lymphocytes and 17±7% monocytes. Nonspecific esterase stains failed to demonstrate esterase activity in any of the cell preparations.

STUDY NO. 2, EX-2. A teflon wound window was fabricated to provide a transparent access port to study growth of bacteria in experimental wounds. This window was implanted into 2 New Zealand White rabbits to see how the animals tolerated the device. One rabbit developed a spontaneous Pseudomonas fluorescens infection while the other developed a mixed infection following implantation of the device. Modification of the window in-house was not possible due to fiscal constraints and lack of manpower. The project was terminated in response to new mission guidelines.

BODY OF REPORT

WORK UNIT NO.	063	Prevention and Treatment of Battlefield Infections
STUDY NO.	1	Establishment of the methods and baseline data required to conduct a normal host immune profile.
	EX-1	Establishment of the capability to perform a host immune profile in Sprague-Dawley rats

PROBLEM

The potential compromise of the soldier's immunity as a result of massive blood loss or resuscitation may increase short or long-term susceptibility to infection and may have major impact on patient management and the time required for soldiers to return to duty. Studies in animals may help identify those areas in which compromise of the immune system can be expected and may indicate management procedures or therapy to minimize the impact of such compromises. Efforts were undertaken to establish appropriate techniques for studying the immune cell functions in rats.

RESULTS AND DISCUSSION OF RESULTS

Histological specimens (liver, spleen, and thymus) were collected from ten rats and evaluated by a veterinary pathologist. All were assessed to be normal. Blood was collected from twenty-five rats and layered over Ficoll-hypaque. Mononuclear cells were collected from the Ficoll-hypaque-blood interface after centrifugation. The cells were suspended at 5×10^6/ml and used in the chemotactic assay. Several substances were used as chemotactants: Zymosan-treated sera (from two separate pools of normal rat serum), filtrate from E. coli cultures, and Salmonella lipopolysaccharide. Smears were prepared and examined after staining with Wright's stain and with nonspecific esterase stain. No consistent increase in the number of mononuclear cells migrating in the presence of any of these substances was seen. Nonspecific esterase stains failed to show esterase activity (α-naphthyl butyrate) in any of the cell populations tested. However, the Wright's stains demonstrated the presence of 17 $\pm$ 7% monocytes in these preparations, and the hemotoxylin stains of the chemotactic filters clearly demonstrated the presence of sufficient numbers of monocytes. The remainder of the cells (83 $\pm$ 7%) were lymphocytes. Large numbers of platelets were present in all preparations.

CONCLUSIONS

Lymphocytes and monocytes can be collected successfully from the blood of rats by layering over Ficoll-hypaque. Zymosan-treated rat sera, E. coli culture filtrate and Salmonella lipopolysaccharide are not good chemotactants for rat monocytes.

RECOMMENDATIONS

Dextran sedimentation of the blood before Ficoll-hypaque separation should lead to high harvests of monocytes. Other stimulants of chemotactic activity should be tried and lymphocyte function evaluated.

PUBLICATIONS

None

STUDY NO. 2	Care and management of contaminated and infected wounds in the combat soldier
EX-1	Development of an animal model for study of wound contamination and infection

PROBLEM

An animal wound model is needed to develop improved methods for treatment in combat casualties. This model should have the following characteristics:

- The animal should respond to wound infection in a manner which would allow the information gathered to be applied to human wound infection.
- A standard wound should be easily created, require a minimum of surgical equipment, and not require prolonged anesthesia.
- The wound model should allow the formation of environments conducive to study both aerobic and anaerobic infections.
- The animal model should provide a wound in which other variables important in the course of wound infection may be studied. Some of the variables are presence or absence of necrotic tissue, clotted blood, and foreign materials.
- The model should allow ready assessment of surgical treatment procedures (debridement, lavage, etc.)

• The wound should be accessible to direct visualization and sampling during the course of infection and treatment. (Other wound models currently in use are closed or covered after inoculation of organisms, are not visualized during the course of infection, and require necropsy for evaluation)

In this study a wound window will be developed for the investigation of infections. Attempts will be made to create reproducible wound infections with species of aerobic and anaerobic bacteria usually associated with wound infections in man.

Due to the unique nature of battlefield environments which may be contaminated by biological, chemical, or radiological warfare agents, and because tactical conditions such as lack of air superiority or interdiction of evacuation routes may dictate excessive delays in delivery of definitive treatment to wounded soldiers, some of the new methods that must be considered may be suboptimal and determinations of efficacy will have to be made under conditions approximating those we may expect to encounter on future battlefields. These features make clinical studies unacceptable. As the amount of information that can be derived from in vitro experiments is limited, reproducible wound infection models in animals will be essential to determinations of feasibility, efficacy, and safety during development of new methods for combat casualty management.

RESULTS AND DISCUSSION OF RESULTS

A 4 cm diameter teflon ring was fabricated. This ring had a wide rim to insert under the skin, and a clear top which could be screwed onto the ring. A second ring with teflon screws was available to slide over the first ring to maintain skin position. This ring was inserted into a male New Zealand White rabbit through a skin incision to the left of the dorsal midline behind the scapula. The window fit well and the animal recovered from the general anesthesia without complications.

The window was tolerated well for 3 days. On the 4th day a thickening of the fascia was noted and a creamy white exudate appeared. The reaction grew progressively worse and the animal was sacrificed on the eighth day after surgery. Microbiological culture of the exudate indicated that it contained a pure culture of *Pseudomonas fluorescens.*

A second rabbit was used to test the window. Within one week of insertion, an exudate developed, although it was not as severe as the one in the first animal.

We feel that the wound window is too heavy and rigid. Additional

fabrication using other materials, such as silastic, in conjunction with testing of the modified devices on normal animals will be needed before any wound infection can be examined. Because LAIR's fabrication facilities are limited, any modification would have to be performed by a private manufacturer. This, coupled with the termination of the wound infection mission in the Division of Cutaneous Hazards, dictates termination of the project.

CONCLUSIONS

Further work is needed to produce an inert wound window for study of wound infections.

RECOMMENDATIONS

This project should be continued in a division at LAIR where investigation of infections can be defended as one of the major problems that must be considered in management of traumatic injuries.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 2392	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	241 APC SL01
b. [redacted]	61102A	3M161102BS02		064
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Analytical Biochemistry Research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002300 Biochemistry; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 09	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	1.6	75
FISCAL YEAR CURRENT 81	6.3	256

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research

ADDRESS:* Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

NAME:* Letterman Army Institute of Research
Division of Research Support

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Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME:* Skala, J.H., DAC

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

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NAME: Waring, P.P., DAC POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Analytical Biochemistry; (U) Instrumentation; (U) Automated Analyses; (U) Clinical Chemistry

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objectives are to develop and adapt new concepts in analytical biochemistry to provide reliable and advanced procedures and services to military-oriented research programs at LAIR and, on occasion, to approved cooperating agencies; to innovate or develop analytical procedures to meet specific needs of research as, for example, the development of micro-automated assay procedures for enzymes related or altered during traumatic or stress conditions; to develop procedures applicable to animal models and human subjects in various research programs and field studies.

24. (U) Analytical support will be provided to studies in military medicine requiring routine analyses in volume or unique equipment and special techniques for assays of physiological specimens obtained during medical research and toxicology projects. Specific analyses will be originated or adapted as required to meet the needs of specific studies and to improve the economy and efficiency of laboratory operations. Research will be conducted on a continuing basis in support of the objectives indicated to provide new methods. Whenever feasible and practical, the methods will be automated and linked to computer systems.

25. (U) 7909-8010. Improved automated methods using continuous flow and discrete analyzers for analysis of aspartate and alanine aminotransferase in micro samples were developed which are applicable to plasma and erythrocytes. An automated continuous flow procedure for erythrocyte transketolase was also developed and is a vast improvement over previous technology. Currently under development are automated procedures for methemoglobin and metmyoglobin reductases and chromatographic methodology for prostaglandins.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M161102BS02 Basic Mechanisms of Recovery from Injury

WORK UNIT NO. 064 Analytical Biochemistry Research

Improved automated methods using continuous flow and discrete analyzers for analysis of aspartate and alanine aminotransferase in micro samples were developed which are applicable to plasma and erythrocyte samples. An automated continuous flow procedure for erythrocyte transketolase was developed and is a vast improvement over previous technology. Currently under development are automated procedures for methemoglobin and metmyoglobin reductases and chromatographic methodology for prostaglandins.

BODY OF REPORT

WORK UNIT NO. 064 Analytical Biochemistry Research

PROBLEM

Ongoing research and other mission-oriented projects require analytical chemistry support. For maximum efficiency of operation, analytical procedures must be simplified and automated. For accuracy and significance to the project, procedural quality with regard to target specificity and interferences must be defined and improved. Frequently, analytical procedures must be developed de novo.

RESULTS AND DISCUSSION OF RESULTS

Despite significant losses of certain pieces of equipment by transfer of function and realignment, the Analytical Chemistry Support Group has retained or acquired a nucleus of automatic analyzers and electronic data processing equipment which provide a generous capacity for routine clinical support to the various studies in which laboratory animals are used. This equipment includes continuous flow analyzers, newly acquired centrifugal and flame photometry automatic analyzers, and a modular laboratory microcomputer.

Greatly improved automated methodology was developed for transketolase, alanine aminotransferase, and aspartate aminotransferase in erythrocytes and the latter two enzymes in plasma. These continuous flow end-point and discrete analyzer kinetic procedures were developed for and applied to several hundred samples collected during an evaluation of the antimetabolite properties of irradiated meat.

An automatic high performance liquid chromatograph (HPLC) with microprocessor assist has been acquired for application to research problems. Preliminary study has begun of procedures for assaying 13,14-diH-15-keto-prostaglandin $F_2\alpha$ in tissues and physiological systems in relation to trauma.

Automated procedures for methemoglobin and metmyoglobin reductases are being developed for application on the centrifugal analyzer. Publication of studies completed during the transition period of the realignment were accomplished this year.

CONCLUSIONS

The automated transketolase and transaminase methods are highly significant improvements compared to previous methods.

RECOMMENDATIONS

Continuous monitoring of operations with the objectives of automating and improving efficiency and quality of performance is required.

PUBLICATIONS

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3. TILLOTSON, J.A., and M.M. BASHOR. Fluorometric apoprotein titration of urinary riboflavin. Anal Biochem 107:214-219, 1980.

4. TILLOTSON, J.A., R.J. O'CONNOR, and E.L. MCGOWN. Ascorbic acid metabolism and body pool size in the monkey. (Abstract) Fed Proc 39:557, 1980.

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6. OMAYE, S.T., J.A. TILLOTSON, and H.E. SAUBERLICH. Metabolism of L-ascorbic acid in the monkey. In: ACS Advances in Chemistry Series, American Chemical Society, edited by P. Seib and B. Tolbert. Washington, DC: American Chemical Society (in press).

7. MCGOWN, E.L. C.M. LEWIS, and P.P. WARING. Investigation of possible antithiamin properties in irradiation sterilized beef. Report No. 71. San Francisco, California: Letterman Army Institute, August 1979.

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9. KNUDSEN, J.J., J.H. SKALA, and H.E. SAUBERLICH. A semi-automated method for the determination of total nitrogen in urine, feces, and diets. Institute Report No. 79. San Francisco, California: Letterman Army Institute of Research, January 1980.

Analytical Biochemistry Research (Cont)

10. TREVINO, G.S., J.H. SKALA, R.S. DEMAREE, J.G. MILLER, B.V. SANDERS, T.A. O'DONNELL, and J.E. CANHAM. Nutrition studies in military German shepherds consuming three commercial rations for thirty-five months with various levels of physical activity. Institute Report No. 83. San Francisco, California: Letterman Army Institute of Research, June 1980.

11. DONG, M.H., E.L. MCGOWN, P.P. WARING, J.H. SKALA, and H.E. SAUBERLICH. Purification of transketolase from human erythrocytes. I. Using solvent denaturation as the initial step. Institute Technical Note No. 13. San Francisco, California: Letterman Army Institute of Research, July 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 2379	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	H.Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS02	00	068
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)*

(U) Coronary Component of Hemorrhagic Shock

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012900 Physiology; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	1.1	51
	CURRENT 81	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Heart Failure; (U) Shock; (U) Coronary Circulation; (U) Vascular Resistance

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Prolonged hemorrhagic shock frequently is refractory to therapy because of failure of the heart. Two hypotheses have been proposed to explain the etiology of the myocardial injury: 1) elaboration by the splanchnic bed of a myocardial depressant, and 2) myocardial ischemia from inadequate perfusion. This study will evaluate the role of the coronary circulation per se in hemorrhagic shock with emphasis on its ability to autoregulate flow.

24. (U) The experimental methodology will involve the construction of diastolic pressure-flow relations during various phases of the shock syndrome in acute and chronically instrumented canine shock preparations. Since normal coronary autoregulation is associated with a characteristic family of pressure-flow relations, departure from this pattern during shock may be indicative of a primary injury at the vascular level. Potentially useful therapeutic interventions will be tested.

25. (U) 79 10 - 80 09 Initial experiments were designed to resolve several conceptual problems dealing with the interpretation of pressure-flow relations and the definition of coronary vascular resistance (see Cardiovasc Res 14:261-269, 1980 for a discussion of these problems). A paper, to be presented at the 1981 FASEB meeting, has been completed which strongly supports the appropriateness of the collapsible tube or waterfall model of the coronary circulation. Although examination of the response of the coronary circulation to hemorrhagic shock in terms of the waterfall model might be expected to give considerable insight, it is not at all clear that this rather considerable effort would result in any change in therapy. Accordingly, persuance of the original protocol is, in the investigator's opinion, not presently indicated. The work unit will be terminated and a new protocol developed to study aspects of coronary physiology and trauma of more direct clinical importance.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3M161102BS02 Basic Mechanisms of Recovery from Injury

WORK UNIT NO. 068 Coronary Component of Hemorrhagic Shock

The following investigations have been conducted under this work unit:

STUDY NO. 1 The effect of coronary sinus occlusion on coronary pressure-flow relations

STUDY NO. 2 Perfused-isolated-arrested heart preparation

STUDY NO. 3 Blood flow during cardiopulmonary resuscitation

STUDY NO. 1. Data exist which have been traditionally interpreted as meaning that the coronary vasculature constricts during at least one phase of hemorrhagic shock. Although the validity of these data cannot be questioned, the conclusion that vasoconstriction has occurred is dependent upon the assumption that the hemodynamic model which was used to perform the data analysis describes physical reality. Vasoconstriction is not directly measured or observed, but is inferred from a calculation of the derived quantity coronary vascular resistance. Several recent papers have suggested that there is an error in the theoretical basis of the presently accepted model used for the calculation of resistance. The purpose of our study is to investigate an alternative hemodynamic model known as the vascular waterfall hypothesis, with the ultimate goal being to study resistance changes during shock. It has been designed to resolve certain conceptual problems such as the relationship between the slope of the coronary pressure-flow relation and vascular resistance. The findings demonstrated that 1) the duration of coronary sinus occlusion is not a factor in determining the effect of coronary venous hypertension, 2) coronary venous pressure is not a determinant of intramyocardial tissue pressure, and 3) the vascular waterfall hypothesis can be used to explain the existence of the atrial cove.

STUDY NO. 2. This study was designed to resolve problems related to the use of coronary pressure-flow relations in the study of shock. Linear coronary pressure-flow relations and a zero flow pressure intercept exceeding venous pressure were found in the potassium-arrested and fibrillating porcine heart. These findings are similar to what has been found in the normal beating dog heart. The zero flow pressure intercept in the fibrillating heart is a function of coronary perfusion pressure since the latter determines the strength of fibrillation. This study has been terminated because clinical relevance is not apparent.

Coronary Component of Hemorrhagic Shock (Cont)

STUDY NO. 3. Blood flow was measured with the radiomicrosphere technique during cardiopulmonary resuscitation in anesthetized pigs. The object of the study was to find a modification of the standard technique for resuscitation that will optimize coronary and cerebral blood flows and would be applicable in the battlefield care of combat casualties. Among potentially useful maneuvers which have been tested and found to be superior to the standard technique are 1) a more rapid rate of chest wall compression, and 2) infusion of epinephrine. Abdominal binding is to be condemned because there is a high incidence of liver laceration. A number of potentially useful interventions remain to be tested.

BODY OF REPORT

WORK UNIT NO.	068	Coronary Component of Hemorrhagic Shock
STUDY NO.	1	The effect of coronary sinus occlusion on coronary pressure-flow relations
STUDY NO.	2	Perfused-isolated-arrested heart preparation

PROBLEM

The behavior of the coronary circulation during hemorrhagic shock is relevant to combat injuries because of incontrovertible evidence that myocardial failure causes death in some experimental shock models. Interpretations of existing data suggest that active coronary vasoconstriction occurs in both early- and end-stage shock. A decrease in coronary blood flow could be an important factor in the development of myocardial failure since contractility is a function of coronary blood flow when perfusion pressure is below 40 mm Hg. The potential for a positive feedback mechanism is apparent since a decrease in myocardial perfusion will result in lower aortic root pressure and a further fall in coronary blood flow. Therapeutic interventions designed to break this vicious cycle have been suggested and are based on the assumption that the coronary vasoconstriction is secondary to alpha sympathetic vasoconstriction or release of vasoactive metabolites such as thromboxane A_2 (TxA_2). Therapy directed toward reducing coronary vascular resistance might be expected to be beneficial, but evidence for its effectiveness is lacking. This discrepancy may result from a fundamental error in the theoretical basis of vascular physiology, an error which causes the coronary pressure and flow data to be interpreted as showing the presence of vasoconstriction. Although the definition of vascular resistance has been accepted for many years, an alternative hemodynamic model based on the hydraulics of collapsible tubes, known as the vascular waterfall hypothesis, has recently been used to explain many aspects of coronary physiology (Cardiovascular Research 14: 261-269, 1980). The purpose of this work unit is to study the coronary circulation during hemorrhagic shock in terms of the vascular waterfall definition of vascular resistance. The experiments were designed to answer the question: Does the coronary circulation respond to hemorrhagic shock by vasoconstriction? Initial studies were designed to resolve certain conceptual problems related to the use of pressure-flow relations and the effect of venous hypertension. Study No. 1 was designed 1) to establish the effect of prolonged coronary sinus occlusion, and 2) to determine the effect of hyperosmolality. Study No. 2 was designed to 1) measure intramyocardial tissue pressure during coronary sinus occlusion, and 2) construct pressure-flow relations in an arrested heart.

Coronary Component of Hemorrhagic Shock (Cont)

RESULTS AND DISCUSSION OF RESULTS

Study No. 1 showed that the duration of coronary sinus occlusion was not an important factor in determining the effect of venous pressure elevation. Furthermore, previous data on the effect of coronary sinus occlusion in the presence of hyperosmolality were shown to be artifactual. It was found that the preparation used in Study No. 1 could also be used to measure intramyocardial pressure. No relation was found between intramyocardial pressure measured with microtransducers buried in the left ventricle and coronary venous pressure except in the fibrillating heart. An incidental finding in Study No. 1 was the effect of atrial systole upon coronary blood flow. These data appear to constitute substantial evidence in favor of the vascular waterfall hypothesis.

Since much of the work planned for Study No. 2 was carried out in Study No. 1, only a few experiments were performed using the protocol of Study No. 2. These experiments showed that coronary pressure-flow relations were linear in the fibrillating and potassium-arrested hearts, and therefore comparable to what is known to exist during diastole in the normal beating heart.

It has become increasingly apparent to the principal investigator that the major direction of these studies is toward basic cardiovascular research which is not likely to have any significant clinical impact and is therefore of questionable military relevance. It may well be that the waterfall definition of coronary vascular resistance is appropriate, but this would probably mean that the coronary bed does not vasoconstrict during hemorrhagic shock and thus there would be no place for any therapeutic interventions designed to modify resistance. There appears to be little justification for continuing a project, the successful completion of which would only form a theoretical explanation for what is already suspected from earlier laboratory and clinical studies.

CONCLUSIONS

Evidence has been found which supports the vascular waterfall model of the coronary circulation. These studies lack clinical and military relevance.

RECOMMENDATIONS

These studies should be terminated. A new work unit has been set up to explore the relationship between trauma and the coronary circulation, which emphasizes clinical relevance.

Coronary Component of Hemorrhagic Shock (Cont)

PUBLICATIONS

1. BELLAMY, R.F., and H.S. LOWENSOHN. Effect of systole on coronary pressure-flow relations in the right ventricle of the dog. Am J Physiol 238: H481-486, 1980
2. BELLAMY, R.F. Calculation of coronary vascular resistance. Cardiovasc Res 14: 261-269, 1980
3. BELLAMY, R.F., H.S. LOWENSOHN, W. EHRLICH, and R.W. BAER. Effect of coronary sinus occlusion on coronary pressure-flow relations in the dog. Am J Physiol 239: H57-64, 1980

STUDY NO. 3 Blood flow during cardiopulmonary resuscitation

PROBLEM

Restoration of coronary blood flow should be the sine qua non of cardiopulmonary resuscitation because, although maintenance of adequate cerebral blood flow is necessary if the quality of post-resuscitation life is to be acceptable, there will be no place for any concern about cerebral function unless the heart is made to function. Little is known about coronary blood flow during open- and closed-chest massage. This is regrettable because several groups have recently proposed modifications of standard closed chest massage which are designed to augment cerebral blood flow which, on theoretical grounds, might be expected to further compromise flow to the myocardium. The purpose of this study is to investigate blood flow to the heart and other organs during cardiopulmonary resuscitation. The efficacy of standard closed-chest massage will be compared to a variety of mechanical and pharmacological maneuvers with emphasis placed upon modifications of the standard technique which are applicable to the battlefield care of combat casualties.

Blood flow will be measured with the radiomicrosphere technique in anesthetized pigs; cardiac arrest will be caused by electrical fibrillation. The efficacy of cardiac massage will be assessed by measuring aortic root pressure, cardiac output, and cerebral, coronary, hepatic, and renal blood flows. The following will be tested:

1. Closed chest cardiac massage according to the standards of the American Heart Association
2. Chest wall compression at 120 compressions per minute
3. Airway pressure of 20 mm Hg for 50% of respiratory cycle
4. Abdominal compression
5. Infusion of intravenous/intraarterial fluid
6. Duration of chest wall compression one-half of massage cycle
7. Open chest cardiac massage
8. Infusion of epinephrine
9. Infusion of the beta endorphin opiate receptor blocker, nalaxone

Coronary Component of Hemorrhagic Shock (Cont)

10. Balloon occlusion of descending aorta
11. Combinations of the above

RESULTS AND DISCUSSION OF RESULTS

Standard closed-chest cardiac massage results in a "cardiac output" of about 20% of normal, with cardiac and cerebral flow being proportionally slightly greater (25-30% of normal). Coronary blood flow is profoundly hindered by the act of chest wall compression (increase in intramyocardial pressure). Coronary perfusion occurs immediately following release of chest wall compression and before aortic root pressure has fallen below 20 mm Hg. A more rapid compression rate increases both cardiac output and organ flow (cerebral and coronary flow 30-50% of normal). Coronary flow is increased even though there is little improvement in blood pressure probably because there are more early "diastoles" per unit time. Neither airway pressure elevation nor infusion of fluid affected measured parameters. Although abdominal compression raised aortic root pressure, its use was associated with a high incidence of liver lacerations. The most effective means found for increasing coronary flow was intravenous administration of epinephrine. Coronary flow frequently exceeded the pre-arrest control, but cardiac output was less than that found with standard techniques (increased afterload?).

CONCLUSIONS

The chest wall compression rate during cardiopulmonary resuscitation should be increased above the currently recommended rate of 60 per minute. Epinephrine should be given whenever possible.

RECOMMENDATIONS

This study should be completed since several promising interventions remain to be tested.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2382	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	EE	249 APC FL 08
b. [redacted]	61102A	3M161102BS02	00	069
c. [redacted]	STOG	80-7.2:1		

11. TITLE (Precede with Security Classification Code)

(U) Physiology of Dermal Penetration

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003200 CBR Warfare; 012600 Pharmacology; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING 80	2.8	108
FISCAL YEAR — CURRENT 81	7.0	128

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

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NAME: White, Charles T., 1LT, MSC, POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Chemical Defense; (U) Skin; (U) Permeability; (U) Dermal; (U) Physiology; (U) Biochemistry; (U) Pharmacology; (U) Pentration; (U) Cutaneous

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Better understanding of metabolic events in skin before and after injury is necessary for development of safe, effective and rational measures to protect soldiers against environmental hazards and for development of decontamination procedures for casualties incurred in a chemical warfare (CW) environment. The objectives of this line of research are: (1) to determine the mechanisms by which various chemical agents produce metabolic aberrations and subsequent tissue damage, and the mechanisms of action of drugs, hormones and other metabolites that may prevent injury, counteract toxic substances, or promote healing; (2) to determine the effects on penetration rates of the physical and chemical properties of the substance, its vehicle and the skin; and, (3) to determine the events occurring in skin during and subsequent to decontamination.

24. (U) Homologous series of chemicals, each series bearing unique chemical groups, will be synthesized and tested for the effects of structures and groups in percutaneous penetration. Skin structure and physiology will be correlated with physiology and mechanisms of skin damage and repair. The mechanisms by which nerve agents and vesicants produce physiologic aberration and tissue damage will be investigated, and the mechanisms of action of therapeutic agents, decontaminants and prophylactic substances on skin will be determined.

25. (U) 79 10 - 80 09. The relevant scientific literature has been reviewed, a research plan has been developed, and protocols for initial research projects have been written. A series of studies has been started to investigate the biochemical alterations induced by an organophosphate simulant of nerve agents, diisopropylfluorophosphate, in the skin and other tissues.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3M161102BS02 Mechanisms of Recovery from Injury

WORK UNIT NO. 069 Physiology of Dermal Penetration

The following investigation has been conducted under this work unit:

STUDY NO. 1 Skin permeability values in model systems and in man

A study has been initiated to compare the skin permeability of various model systems (in vitro, hairless dog, weanling pig) to that of man. An in vitro apparatus developed for the study of topical mosquito repellents is being adapted for the study of skin evaporation and penetration of chemicals in general. It presently has the capability to operate with any skin type, has controlled variable air flow above the skin and fluid circulation and temperature below the skin, can trap volatile substances evaporating from the skin with no dead space, and will allow variation in the temperature and humidity of incoming air to affect changes in stratum corneum hydration and temperature. Modifications will increase the mechanical reliability and allow for more replicates to be completed in the same amount of time.

BODY OF REPORT

WORK UNIT NO. 069 Physiology of Dermal Penetration

STUDY NO. 1 Skin permeability values in model systems and in man

PROBLEM

Healthy normal skin functions primarily as an organ of protection and helps maintain homeostasis. Because the skin is an organ in direct contact with the environment, it is susceptible to chemical insults. On the modern battlefield, the soldier may be exposed to chemical warfare attack, and his skin may be contaminated with chemical agents. Casualties appearing at medical treatment facilities are likely to have sublethal amounts of agent(s) on their skin. Research is needed to develop quantitative methodology to measure sublethal levels of chemicals on or in the skin. This technology can then be used to assess degrees of contamination and efficacy of decontamination as part of the development of decontamination technology. In addition, this research effort, by defining models of loss of chemicals from the skin surface, can support efforts to improve the persistence of desirable chemicals on the skin surface, such as mosquito repellents.

In the study of mechanisms underlying some of the phenomena in environmental skin disease, information has been collected on percutaneous absorption of a number of drugs and simple chemicals, but not on most environmental penetrants. Research on the barrier function of skin and molecular penetration has been meager. Systematic development of models for the study of percutaneous absorption is needed. The effects of a host of variables (skin hydration, organic solvents, molecular structure and physical properties of penetrating molecules, etc.) on skin penetration need further study. With this information, new methods could be developed to prevent penetration of toxic chemicals into the skin.

Various in vitro and animal models for determining percutaneous penetration have been developed in recent years at LAIR. Most of these procedures were developed to support the mosquito repellent development program. Nevertheless, they may be employed for studying other chemicals, and they have proved to be adapted easily to meet new requirements, as evidenced by the success in providing systems data to support the USAF Litter Patient Shower Decontamination project (Institute Report No. 86, Letterman Army Institute of Research). The in vitro models employ epidermis, stratum corneum, or whole skin from man or animals. They offer

greater control of experimental variables than can be achieved in vivo. There is no experimental evidence that the barrier function is altered after excision of skin shortly after death. If properly stored in the frozen state, the horny layer maintains its essential properties over several months. Nevertheless, the anatomic site from which the skin was taken must be considered, and variables associated with the site (temperature, degree of hydration) should be reproduced in the model.

The animal models allow investigation of the dynamic processes that depend on a living dermis. Thus, for experiments where enzymes are to be studied or where a dynamic microcirculation is to be investigated, a living model is required.

This study is addressing the comparability of various animals' skin permeability to that of man and the comparability of in vitro and in vivo skin permeability values, as it is unlikely that a single species will be adequate for all needs in either type of system. Therefore, several compounds with reported values for percutaneous penetration in man will be tested on the pig and the hairless dog to evaluate the models. The in vitro model will be modified to make it more mechanically reliable and easier to use for studying percutaneous penetration and evaporation from the skin. The effects of variations in fluid circulation and temperature below the skin, and flow rate, temperature and humidity of incoming air above the skin on percutaneous penetration will be determined with pig skin and compared to in vivo measurements to establish standard settings for the variables. Then, the in vitro permeabilities of reference compounds will be determined using pig skin and cadaver skin.

RESULTS AND DISCUSSION OF RESULTS

Based on results from previous studies of the interaction of chemicals with the skin, an overall 5-year effort was planned to define physical, chemical, and biological mechanisms important in the protection and maintenance of skin integrity and function. A study has been initiated to compare skin permeability in various models and man. The information derived supports efforts in chemical defense and formulation of improved mosquito repellents.

CONCLUSIONS

None

RECOMMENDATIONS

None

Physiology of Dermal Penetration

PUBLICATIONS

REIFENRATH, W.G., P.B. ROBINSON, V. BOLTON and R.E. ALIFF: Percutaneous penetration of mosquito repellents in the hairless dog - the effect of chemical dose on percent percutaneous penetration. Food Cosmetic Toxicol (in press)

REIFENRATH, W.G. and P.B. ROBINSON: Evaporation and penetration characteristics of mosquito repellents using an in vitro model. (submitted for review and clearance)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE6104	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AD	082 JL02
b. ~~CONTRIBUTING~~	61102A	3M161102BS02		074
c. ~~CONTRIBUTING~~	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Long Term Cryopreservation of Platelets for Immediate Field Use

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 012900 Physiology; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 01	80 10	DA	C. IN-HOUSE

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	3.6	117
FISCAL YEAR CURRENT 81	2.6	74

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Cheney, Barbara A., MS, DAC

NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Platelet Storage; (U) Cryopreservation: (U) Blood Storage; (U) Massive Transfusion; (U) Platelet Transfusion; (U) Traumatic Hemorrhage

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The need for effective hemostasis in severe combat injuries requires the availability of timely, effective component hemotherapy with coagulation factors and platelets. The former can be provided with relative ease but the latter, being perishable (72 hour storage limit) are logistically difficult to provide to rear line (even CONUS) medical facilities, and even more so to forward resuscitation units. This study is designed to develop and test storage systems whereby effective clinical doses of platelets can be stored, frozen for long periods of time, then easily thawed ready for immediate or delayed transfusion.

24. (U) The objectives of this work are to develop feasible freezing techniques using in vitro and in vivo tests of platelet viability and function to determine storage induced cellular injuries; evaluate existing full size clinical freezing protocols as to military objectives, feasibility and necessary modifications; develop therapeutic dose single unit capability; develop post-thaw suspension medias whereby platelets can be stored beyond 24 hrs; evaluate clinically feasible products in vivo on humans; evaluate in vitro tests of platelet function and viability and correlate to in vivo results to develop a battery of in vitro tests for pre-clinical studies.

25. (U) 79-10--80-09 a. Clinical trials were performed with human volunteers to evaluate glycerol-glucose cryopreserved platelets. This protocol showed promise as a no-wash post-thaw technique but both static rate freezing (N=5) and controlled rate freezing (N=7) had in vivo platelet recoveries below 20%. b. Pheresis harvested platelets were collected and stored for two weeks in one bag. Recoveries (in vitro) were 50% of harvest suggesting storage conditions are not optimal. c. Density separation studies on liquid stored platelets showed cells can be separated according to degree of storage injury and glycoprotein membrane changes can be determined.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3M161102BS02 Basic Mechanisms of Recovery from Injury

WORK UNIT NO. 074 Long-Term Cryopreservation of Platelets for Immediate Field Use

The following investigations have been conducted under this work unit:

STUDY NO. 1 Cryopreservation strategies

STUDY NO. 2 In vitro viability function testing

STUDY NOS. 1 and 2. Severe injury to combat soldiers requires large volume fluid therapy to sustain life. In this setting, clotting factors and platelets are depleted through losses in shed blood, consumption, and dilution due to transfusion, all of which act in combination to impair hemostasis. The hemostatic defects can be corrected with transfusions of plasma (rich in coagulation factors) and platelet concentrates. Since platelets for transfusion must be frozen for long-term storage to meet military logistical requirements, this division addresses practical methods whereby platelets can be easily frozen, stored for long periods, thawed, and made ready for immediate or delayed use. This strategy places emphasis on preparing a one unit therapeutic dose that can be processed with minimal delay after it is thawed. Phase I clinical trials, in conjunction with Letterman Army Medical Center's Clinical Investigation Service, were performed with a freezing protocol (4% glycerol-5% glucose as the cryoprotectant) that fulfilled the military strategy requirements. This evaluation revealed that although the protocol fulfilled logistical needs, the in vivo recoveries were inadequate to fulfill therapeutic needs. Techniques to evaluate platelet storage changes in vitro have been developed in this laboratory and are being correlated with in vivo viability. Transfused platelet recovery can be accurately predicted by these in vitro tests.

BODY OF REPORT

WORK UNIT NO.	074	Long-Term Cryopreservation of Platelets for Immediate Field Use
STUDY NO.	1	Cryopreservation strategies

PROBLEM

Massive transfusion of stored blood or blood substitutes following severe combat injuries leads to impaired hemostasis. This defect aggravates bleeding, and leads to an inability to resuscitate the wounded soldier successfully. The defect is due to many factors: trauma, dilution of blood with resuscitation fluids, and the lack of platelets and coagulation factors in stored blood products. Platelets can be prepared and given in massive transfusion situations to prevent and treat bleeding due to thrombocytopenia. Blood and coagulation factors are relatively easy to obtain and store for massive transfusion needs but platelets stored in conventional liquid storage systems are too perishable (72 hr storage period) for field use. Current freezing schemas for storing platelets are cumbersome and time-consuming. The platelets require extensive washing after thawing to eliminate possible toxic cryopreservatives and the procedures are not field adaptable. This study is aimed at evaluating simple cryopreservation processes in terms of the field adaptability as well as storability for 72 or more hours after thawing.

RESULTS AND DISCUSSION OF RESULTS

A cryopreservation protocol, based on the work of Drs. Pert and Dayian of Albany, New York, has been established. The cryoprotectant in this protocol is 4% glycerol and 5% glucose. Since these compounds are physiological in the final product (1% less than each glycerol and glucose), the procedure does not require extensive processing after the platelets are thawed, it requires only dilution of the platelets with acidified plasma. Tests in our laboratory show this procedure results in a product with acceptable in vitro recovery after freezing. In addition to in vitro studies, a protocol was established with Letterman Army Medical Center's Clinical Investigation Service to evaluate the product of the glycerol-glucose cryopreservation protocol in vivo. Normal volunteers (N=12) were given autologous s thawed platelets labelled with ^{51}chromium. Two freezing techniques for the platelets have been used: controlled rate (33 C/min) and static rate (liquid nitrogen plunge). Those platelets frozen by controlled rate (donors N=5) had in vitro recoveries of 17±9% whereas those frozen by static rate (donors N=7) had in vitro recoveries of 72±9% with in vivo recoveries of 20±4%. Both groups had normal in vivo lifespans (7.2±1.1 versus 8.4±1.7 days). These results show that static rate freezing is better than controlled rate freezing because of higher in vitro recovery. Static rate techniques are simple and adaptable to military needs. Unfortunately, the in vivo recoveries are less than current cryopreservation

strategies (using DMSO as the cryoprotectant, recoveries are reported in the literature at 35-40%). Function of previously frozen platelets is currently being evaluated in thrombocytopenic patients. At the present time two non-immunized patients, who have shown good response to previous platelet transfusions, have been given therapeutic doses (> 3.3×10^{11}) of glycerol-glucose preserved frozen-thawed platelets. One patient developed a fever after transfusion (103 F) but did not have a rise in the platelet count or a shortened bleeding time. The other patient did not have a febrile response; neither did this patient have a rise in platelet count or shortened bleeding time. These results suggest the frozen platelets are available and may not be functional.

CONCLUSIONS

Although the glycerol-glucose protocol fulfills military logistic needs, the in vivo studies do not support a conclusion that the procedure is adaptable for therapeutic needs for thrombocytopenic patients.

RECOMMENDATIONS

The glycerol-glucose procedure, as designed, is inadequate and, therefore, a revision should be made so that further studies address optimization of yields after freezing of the platelets. Unless in vivo yields are greater than 30%, this procedure will not become therapeutically useful. Further investigation into the use of simple platelet harvest from donors and commercially adaptable blood platelet plastic bags (e.g. polyvinyl chloride) will also be needed to optimize military adaptability of glycerol-glucose protocol.

PUBLICATIONS

None

STUDY NO. 2 In vitro viability, function testing

PROBLEM

The development of frozen platelet protocols has had to rely on the ability to evaluate platelets by in vitro parameters. The tests currently available have not been reliable from laboratory to laboratory and have questionable value when the platelets are perturbed by the presence of cryoprotectants.

Long-Term Cryopreservation of Platelets for Immediate Field Use

RESULTS AND DISCUSSION OF RESULTS

Tests of platelet integrity and autologous function have been performed on platelets frozen, thawed then infused into the donor. Morphology score appears to be the best indicator of in vivo platelet recovery. Actual recovery, in vitro, does not correlate with in vivo results. Osmotic shock recovery was too insensitive a test to evaluate in vivo results. The in vivo recovery was measured by radiolabel techniques (^{51}Cr) in which labelling was done after thawing the platelets. This procedure, as compared to labelling the platelets before freezing may bias the results so that in vivo correlations cannot be accurately made.

CONCLUSIONS

Tests of platelets based on morphology are valid for predicting in vivo recovery.

RECOMMENDATIONS

Morphology tests should be used to evaluate cryopreserved platelets to determine the ability of these cells to tolerate freezing. Multiple variable analysis should be made on all tests to see if in vitro observations can be strengthened.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OG 2371	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	K.COMPLETION	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS02	00	077 APC 503C
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Irradiated Food Antimetabolite Study

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

006500 Food; 002300 Biochemistry; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 11	80 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	4.2	173
FISCAL YEAR CURRENT 81	0.0	0

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Fruin, J.T., LTC, VC

NAME: Lewis, C., DAC POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Irradiated Chicken; (U) Anti-Vitamin B_6; (U) Blood Aminotransferases; (U) Vitamin B_6

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The Food and Drug Administration requires the conduct of various animal feeding test to demonstrate and ensure the safety of irradiated foods prior to submission and approval of an Army petition for consumption of irradiated meats by the military personnel. The required animal tests were outlined in a protocol entitled "Animal Feeding Protocol for Irradiation Sterilized Test Foods" originated by the Office for the Wholesomeness of Irradiated Foods, USAMRDC, dated 21 Oct 1975. The objective of this study was to determine whether irradiation, freezing, or thermal processing of chicken produces factors which are antagonistic to pyridoxine in the diet of rats.

24. Male and female rats were fed a diet devoid of vitamin B_6 until they were judged deficient according to a pre-determined weight gain criterion. They were then randomly divided into groups and repleted with diets containing chicken which had been preserved by freezing, thermal processing, gamma or electron irradiation. Each diet was fed at two levels of pyridoxine to determine whether any anti-vitamin substance (if present) could be overcome by additional vitamin. Recovery rates were monitored by growth responses and blood aminotransferase activities (enzymes which require pyridoxal phosphate for activity).

25. (U) 7911-8009. Study completed. No differences were observed in weight gain among the chicken-fed groups. The enzyme responses of rats fed frozen, thermally processed, or electron irradiated chicken were similar. Responses of some of the enzymatic parameters were slightly delayed in groups fed gamma irradiated chicken (at marginal vitamin level only). If any anti-vitamin B_6 factor is present in gamma irradiated chicken, it is minimal, is detectable only under conditions of marginal vitamin B_6 status, and is overcome by added dietary pyridoxine.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO.	3M161102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	077	Irradiated Food Antimetabolite Study

The following investigation has been conducted under this work unit:

STUDY NO. 1 Irradiated food antimetabolite study

Male and female rats (156 each) were made vitamin B-6 deficient by feeding a semi-purified diet devoid of vitamin B-6. They were then repleted with various test diets containing chicken which had been preserved by one of four methods: frozen, thermally processed, electron or gamma irradiated. All repletion diets contained carefully controlled (marginal or high) levels of vitamin B-6. Recovery rates were monitored by growth (weight gain) and measurements of vitamin B-6-dependent blood enzymes (plasma and red cell aspartate aminotransferase and alanine aminotransferase). No differences were observed in weight gain among the chicken-fed groups. The enzyme responses of rats fed frozen, thermally processed or electron irradiated chicken were similar. Responses of some of the enzymatic parameters were slightly delayed in the groups fed gamma irradiated chicken at the marginal vitamin level. No consistent differences were observed between any of the high vitamin groups. If antivitamin B-6 factor is presented in gamma-irradiated chicken, it is minimal, is detectable only under conditions of marginal vitamin B-6 status, and is overcome by added dietary pyridoxine.

BODY OF REPORT

WORK UNIT NO.	077	Irradiated Food Antimetabolite Study
STUDY NO.	1	Irradiated food antimetabolite study

PROBLEM

The goals of the U.S. Army irradiated food program, initiated in 1954, were to develop the technology and establish the wholesomeness of foods which have been sterilized by irradiation. The advantages of preservation by irradiation include platability (compared to canned or dried foods) and substantial savings in distribution and storage costs by eliminating the requirement for refrigeration. Such preservation would simplify logistical support problems through reduction of the requirement for refrigerated ships, trucks, and deep-freeze lockers to handle perishable foods such as meat, fish, and poultry. It would also be beneficial in the storage of nutritious rations as part of a war time civil defense program or to meet emergencies such as natural disasters.

Despite extenxive testing by scientists throughout the world, no harmful effect has been found in animals which have consumed irradiated foods. Many countries have already approved a variety of irradiated foods for unlimited human consumption on the basis of these exhaustive tests (Bull. Atomic Scientists, 34:50-55, 1978). However, the U.S. Government considers irradiation an additive rather than a process, and it is, therefore, subject to the requirements of the 1958 Food Additive Amendment to the Federal Food, Drug and Cosmetic Act before approval can be granted. Additionally, the FDA has required that tests be conducted to ascertain whether or not irradiation produces substances which are antagonistic to certain vitamins, notably thiamin and vitamin B-6. The study described below was designed to test for antivitamin B-6 properties in irradiation sterilized chicken.

RESULTS AND DISCUSSION OF THE RESULTS

Male and female rats were red a semipurified diet devoid of vitamin B-6 until they became deficient according to a preset weight gain criterion. They were then repleted with semipurified diets or diets containing chicken (frozen, thermally processed, gamma or electron irradiated). Each diet was fed at two levels of pyridoxine, a high level (12.0 mg/kg) and a marginal level (2.5 mg/kg). The high vitamin groups were included to determine whether any antivitamin substances (if present) could be overcome by extra pyridoxine. Recovery parameters were weight gain and several blood aminotransferases (which require pyridoxal phosphate for activity). These included plasma and

erythrocyte aminotransferase and alanine aminotransferase.

No differences were found in growth response among any of the chicken-fed groups. The groups fed semipurified diets responded slower, presumably because they consumed less food than the rats fed chicken diets.

The blood aminotransferases responded similarly in the groups frozen, thermally processed, and electron irradiated chicken. Thus, these three diets were considered equivalent in terms of vitamin B-6 availability. Responses of some of the enzymatic paramaters were slightly delayed in groups fed gamma irradiated chicken at the marginal vitamin B-6 level. Although these differences were statistical, their magnitudes were small compared to the overall responses to repletion. However, these results could be interpreted as a slightly lower availability of vitamin B-6 in the gamma irradiated (low vitamin) chicken diet. No differences were observed at the high vitamin level.

CONCLUSIONS

Although irradiation does cause some destruction of vitamin B-6, the loss is similar to that observed after thermal processing (canning), and there is no major production of antivitamin substances. No evidence was found for antivitamin B-6 properties in electron irradiated chicken. Gamma irradiated chicken may have a slightly reduced vitamin B-6 availability, but it is minimal and probably not important enough to offset advantages of food preservation by irradiation.

RECOMMENDATIONS

Irradiation-sterilized chicken should be considered safe for human use with respect to vitamin B-6 availability. Such chicken consumed in a normal diet would have no detrimental effect on vitamin B-6 nutritional status.

PUBLICATIONS

1. RAICA, N., JR., E.L. MCGOWN, and D.E. HILMAS. The absence of antithiamin factors in radappertized beef and chicken. In: Proceedings, Vol. 1, 26th European Meeting of Meat Research Workers, (Colorado Springs, CO, 31 August-5 September 1980), pp 229-231

2. FRUIN, J.T., C.D. KUZDAS, and L.S. GUTHERTZ. Mutagenicity studies with irradiated meats. In: Proceedings, Vol. 1, 26th European Meeting of Meat Research Workers (Colorado Springs, CO, 31 August-5 September 1980), pp 241-244

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 3371	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 07 15	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	243 APC HL19
b. [redacted]	61102A	3M161102BS02	00	078
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Ballistic Injuries

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 008800 Life Support; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 08	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	0.1	16
FISCAL YEAR CURRENT 81	7.0	231

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME: Belkin, Michael

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Wound Healing; (U) Military Trauma; (U) Animal Model; (U) Laser

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Data exist suggesting that the laser scalpel facilitates the debridement of third degree burns. It is not known if a similar benefit is found when the laser scalpel is used to debride penetrating soft tissue wounds.

24. (U) A captive bolt gun will be used to create a standard soft tissue wound in anesthetized pigs. Wounds will be debrided using either 1) cold knife, 2) electro-cautery, or 3) argon laser-assisted quartz scalpel. The three methods of debridement will be compared for efficacy and magnitude of blood loss.

25. (U) 80 08 - 80 09 It has been demonstrated in a pilot project that the captive bolt gun firing a drive pin with maximum powder charge causes a ballistic injury which can serve as a model for debridement. A suitable laboratory space is being modified to allow the installation of the necessary laser equipment.

*Available to contractors upon originator's approval.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3M161102BS02 Basic Mechanisms of Recovery from Injury

WORK UNIT NO. 078 Ballistic Injuries

The following investigation was conducted under this work unit:

STUDY NO. 1 Ballistic injury using captive bolt gun

An anesthetized pig model will be developed to study three surgical modalities used in the debridement of soft tissue wounds. Wounds will be made using a captive bolt gun and will be debrided with either 1) cold knife, 2) electrocautery, or 3) argon laser-assisted quartz scalpel. Results will be determined by measuring 1) the amount of bleeding, 2) speed of debridement, 3) amount of tissue removed, and 4) iatrogenic tissue injury. This is a new protocol and no data have been collected.

BODY OF REPORT

WORK UNIT NO.	078	Ballistic Injuries
STUDY NO.	1	Ballistic injury using captive bolt gun

PROBLEM

The debridement of soft tissue wounds caused by high velocity missiles may cause considerable blood loss in casualties already hypovolemic. Data exist showing that the argon laser-assisted quartz scalpel is superior to the traditional cold knife from the standpoint of blood loss and speed when used to debride burn eschars. We need to determine whether or not the laser scalpel has similar value when used to debride soft tissue wounds. Since the LAIR ballistic laboratory is not yet complete, pursuance of this goal will require use of an alternative method of creating a model ballistic injury. Even though it is a low velocity missile, the drive pin of a captive bolt gun (probably because of its irregular shape) can cause considerable destruction when fired into soft tissue. Anesthetized pigs will be shot in their posterior thighs and debridement performed after one hour. Three surgical procedures will be compared: 1) cold knife, 2) electrocautery, and 3) laser scalpel. Criteria of efficacy will be 1) amount of bleeding, 2) speed of debridement, 3) amount of tissue removed, and 4) iatrogenic tissue injury.

RESULTS AND DISCUSSION OF RESULTS

This is a new protocol. Only the feasibility of using the captive bolt gun has been demonstrated.

CONCLUSIONS

This model is feasible.

RECOMMENDATIONS

The present protocol should be followed and the studies should be implemented.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OG 2373	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM'RY
79 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	CA	201 APC FL07
b. [redacted]	62770A	3M162770A802	00	122
c. [redacted]	STOG	80-7.2:2		

11. TITLE (Procede with Security Classification Code)*

(U) Development of Repellents Against Medically Important Arthropods

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology; 002300 Biochemistry; 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	84 06	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	6.9	
FISCAL YEAR CURRENT 81	4.8	165

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Buescher, Michael D., 1LT, MSC, POC:DA

21. GENERAL USE

Foreign Intellignece Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Controlled-release; (U) Formulations; (U) Evaporation; (U) Absorption; (U) Insects; (U) Arthropods; (U) Vectors; (U) Protection; (U) Repellents

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Repellents provide the broadest spectrum of protection, so they are the most cost-effective means for protecting against biting arthropods and the diseases they carry. Safer, longer lasting, more effective and more pleasant repellents are needed to replace present items of issue. Present knowledge and technology makes replacement feasible within 5 years.

24. (U) Candidate repellents will be evaluated against a battery of medically important species. Controlled-release formulations will be evaluated for troop acceptability and duration of efficacy. Area repellents will be tested. Toxicological testing will be performed as required to establish safety and environmental impact.

25. (U) 79 10 - 80 09. Dimethylphthalate, Indalone™ and Citronyl™ were found to be suitable interim commercially available repellents for use if EPA revokes registration of diethyltoluamide (deet). A colony of chigger mites is being established for repellent testing. The list of new candidate repellents under active consideration was reduced to 5, which have been referred to toxicology and advanced entomological testing. Two silicone polymer formulations of deet were found to provide significantly longer protection vs mosquitoes than other deet formulations. New and more accessible field test sites were established in the San Francisco bay area. Results of field tests on commercially available area repellents and on new candidate formulations prepared in-house were inconclusive, but gave no indication that further testing would be warranted. Therefore, the area repellent testing program is being discontinued.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3M162770A802 Military Preventive Medicine

WORK UNIT NO. 122 Development of Repellents Against Medically Important Arthropods

Two silicone polymer formulations of diethyl toluamide (deet) were significantly superior to the unformulated compound when tested on mice against the mosquito *Aedes aegypti*. Promising new repellents of the Stanford Research Institute and the U.S. Department of Agriculture were tested against representative species of *Aedes*, *Culex*, and *Anopheles*. Five repellents were selected for toxicological evaluation and further testing against sand flies, fleas, ticks and bugs. Dimethyl phthalate, Indalone™ and Citronyl™ (R-69) were recommended as replacements for deet if registration of the latter is canceled on short notice.

BODY OF REPORT

WORK UNIT NO. 122

Development of Repellents Against Medically Important Arthropods

PROBLEM

Repellents supplement pesticides, physical barriers, vaccines and drugs in limiting the impact of biting arthropods, and the diseases they carry, on the will and the ability of soldiers to fight. However, the repellents currently issued by the Army are not effective against all species of insects or under all conditions of weather and climate. They are not well-liked by field troops. Usage in Vietnam was not sufficient to prevent heavy losses of manpower from vecto. borne diseases. A localized, severe, primary reaction to diethyl toluamide (deet) was observed among soldiers in Vietnam. Spermatotoxic and embryotoxic effects of deet have been observed in experimental animals. The safet; of deet for human use is currently being reconsidered by the Environmental Protection Agency (EPA), and it is possible that its registration may be eventually canceled. Research under Work Unit No. 122 is directed toward the development of improved formulations of deet to provide better performance, greater safety and increased troop acceptance, and toward the identification of acceptable substitutes and/or superior compounds to replace deet. The development and testing of materials which may be useful as area repellents are also included in the research program.

RESULTS AND DISCUSSION OF RESULTS

Deet is currently issued in the military as the 75% solution in ethanol. However, certain formulation additives have the potential to reduce absorption and evaporation of deet from the skin, thereby increasing its period of effectiveness on the skin and reducing the systemic hazard from absorption. Such additives may also enhance the user acceptability of deet by improving its spreading properties, "feel," and other subjective qualities. Twenty-two formulations of deet incorporating silicone, acrylate and latex polymers were prepared at LAIR and two formulations incorporating commercial copolymers were obtained from Bend Research, Inc. Each formulation was tested against the yellow fever mosquito, *Aedes aegypti*, in an in vitro test system (ED_{50}) and on white mice (4-hour ED_{50}) to compare the effectiveness of the formulated deet with that of unformulated deet on an equimolar basis. Two of the formulations prepared at LAIR were significantly superior by both test methods. These formulations will be tested on volunteers in FY 81 if human-use trials are approved. The additives are clear, water-repellent, nontoxic silicone polymers used in cosmetics, skin preparations, processed foods and other commercial products.

SRI 434-58	Methyl-N,N'-di-(n-hexyl)ethlene-diamine-mono-carbamate
USDA A13-36166	(E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline
USDA A13-36178	1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl) quinoline

Currently, toxicity data on these five compounds are limited, but favorable. Further toxicity testing of SRI 835-23A is now in progress in the Division of Research Support, LAIR, and tests on human volunteers will be conducted in FY 81 following human use approval. Adequate test quantities of SRI 835-23A are currently on hand, and additional amounts of SRI 835-19C, SRI 434-58, USDA A13-36166, and USDA A13-36178 have been placed on order. Tests on experimental animals against sand flies, fleas, ticks, and bugs were initiated during the year.

The development and testing of area repellents for protection of troops in bivouacs, outposts, entrenchments and similar situations were initiated in FY 78. Field trials of two experimental materials and a commercial product conducted at Colusa, California, in FY 79 were inconclusive albeit the latter material had been highly effective in independent trials conducted in New York State in 1972. The reason for the discrepant findings is not known, but it may be related to the differences in climate, mosquito test species, or methods of collecting mosquitoes in the test area. New tests designed to resolve this problem and to settle the area repellent question will be conducted in late FY 80 or FY 81.

Improvements in "materials and methods" are made on a continuing basis as an integral part of the program of repellent research at LAIR. During FY 80, two colonies of mosquitoes (*Culex pipiens* and *Anopheles quadrimaculatus*) and a colony of bugs (*Triatoma barberi*) were discontinued as excess to current needs. A colony of chigger mites (*Leptotrombidium fletcheri*) was obtained from the Walter Reed Army Institute of Research for use in advanced testing of new repellents. The battery of test species currently maintained includes four species of mosquitoes, a sand fly, a flea, two species of ticks, a bug, and a chigger mite. Trials of common laboratory animals as repellent test subjects, originally instituted in FY 73, were discontinued in FY 80. Of the species considered (hairless dogs, white mice, white rabbits and guinea pigs), none was superior as a model system, but white mice and rabbits were more practical and economical to use. Comparative trials of the "no choice" and "free choice" designs for bioassay of repellents were begun in FY 80. Preliminary results indicate that the two methods give essentially similar results. The "no choice" design is, strictly speaking,

Development of Repellents Against Medically Important Arthropods

Eight commercial repellents have been evaluated as "fallback" replacements for deet since 1976. Testing of these materials was increased in FY 80, and nearly complete data on their effectiveness against two species of mosquitoes, a sand fly, a flea, and two species of ticks are now available. On the basis of these data, three of the eight appear to be superior as general purpose repellents: (1) dimethyl phthalate was developed by the Standard Oil Development Company in 1929, and it is still used in some commercial products. It was used extensively by the Army in World War II, and is credited with substantial success in preventing losses to malaria, dengue, and scrub typhus. It is reported to be as effective as deet against certain mosquitoes and deer flies. (2) Indalone™ was developed by the Kilgore Development Corporation in 1937, and it also is still used in commercial formulations. It has been reported to be just as effective as deet against sand flies, and this is borne out in the tests conducted at LAIR. (3) Citronyl™ (R-69) is a product of S.C. Johnson & Son, Inc. It is currently sold in Canada but is not yet registered for use in the U.S. Our tests indicate that it is approximately equivalent to deet as a repellent, but the USAEHA has reported that it can cause injury to the cornea and conjunctiva. None of the foregoing repellents is as persistent on the skin as deet, and if one is needed by the Army in the future, a controlled-release formulation should be developed for it as indicated above for deet.

A further finding in our evaluation of commercial repellents was that ethyl hexanediol, known as Rutgers 612, is comparatively ineffective as a repellent despite its apparent commercial success. This repellent is issued by the Army in stick form, compounded with stearic acid and wax. The need for the stick repellent should be re-examined and we should consider another active ingredient to substitute for the ethyl hexanediol.

The eventual replacement for deet should be a new compound that is more effective than deet, more persistent on the skin, and both nontoxic and pleasing to the user. During FY 76 to FY 79, 120 new compounds obtained from the Stanford Research Institute (SRI) and the U.S. Department of Agriculture (USDA) were tested against the yellow fever mosquito at LAIR. During FY 77 to FY 80, selected compounds of this group were further tested against *Aedes taeniorhynchus*, *Culex pipiens*, *Anopheles albimanus*, and *Anopheles stephensi*. Twenty-seven compounds were retained on the basis of these tests, but ten of them, including N-(n-hexyl)-2-oxazolidine, mentioned by name in last year's report, have now been excluded on the basis of toxicity data obtained from SRI, the USAEHA, and the Division of Research Support, LAIR. Of the 17 remaining compounds, the following are believed to be the most promising:

SRI 835-23A	N(n-octyl)glutarimide
SRI 835-19C	N(n-hexyl)glutarimide

AD-A122 728 ANNUAL RESEARCH PROGRESS REPORT FY 1980(U) LETTERMAN 2/3
ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA
J D MARSHALL 01 OCT 80

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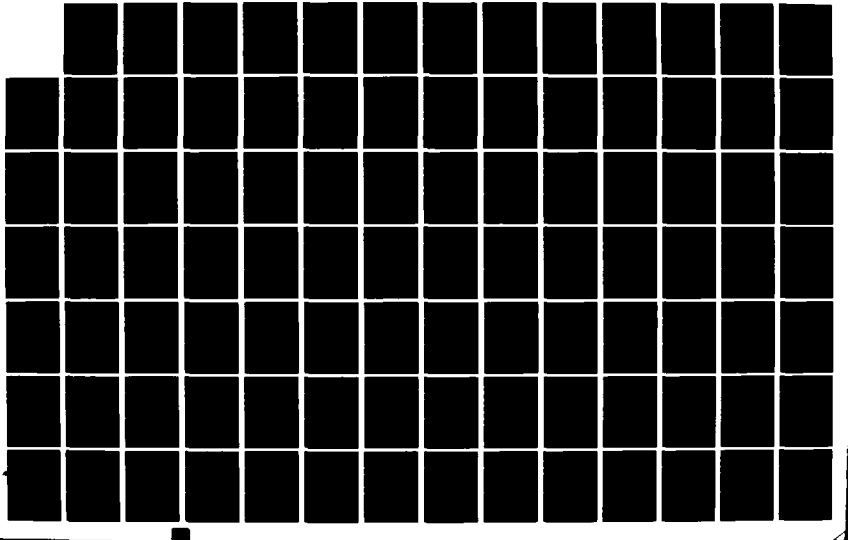

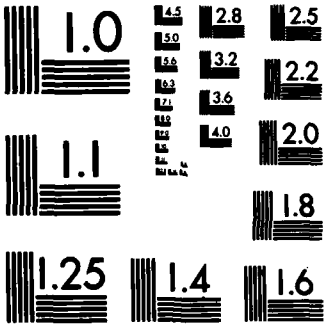

more appropriate for the probit, logit and other classical methods of statistical analysis. The "free choice" design is more natural and, it is the only one possible in tests against free-ranging insects in the field. In this connection, an additional field testing site in the San Francisco Bay area was identified and mapped by CPT John C. Owens, MS, (LAIR mobilization designee) during this fiscal year. Easily accessible sites are needed in the field testing phase of the repellent development program.

CONCLUSIONS

Prospects for the continued use of deet by the military are presently uncertain. If deet is reregistered by the EPA, development of new film-forming polymer formulations of deet may be more immediately practicable than the fielding of an entirely new compound. Adoption of a new compound will be desirable however, if one is eventually shown to be superior to deet and safe for troop use. If deet is cancelled by the EPA, a switch to one of the new compounds or to one of the older less persistent materials will be imperative. The repellent should be formulated in conformity with modern principles of controlled release in either case.

RECOMMENDATIONS

Current studies in the area of controlled-release technology for insect repellents should be extended to tests on human volunteers. Toxicity testing of the new compounds that have been identified as possible replacements for deet should be expedited so that their status can be conclusively determined. The uncertain results obtained to date in field tests of area repellents should be followed up and clarified because of the great potential benefit of area repellents to the Army.

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WIRTZ, R.A., J.D. TURRENTINE, and L.C. RUTLEDGE. Area repellents for mosquitoes (Diptera: Culicidae): Laboratory testing of candidate materials against *Aedes aegypti*. Mosq News 40: 432-439, 1980

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SPENCER, T.S., J.A. HILL, R.J. FELDMANN, and H.I. MAIBACH. Evaporation of diethyltoluamide from human skin *in vivo* and *in vitro*. J Invest Dermatol 72:317-319, 1979

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HOOPER, R.L. Prevention and control of medically important arthropods. In: Maxcy Rosenau's Preventive Medicine and Public Health, 11th ed., edited by J. Laft. New York: Appleton-Century-Crofts. (In press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6079	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	H. Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3E162772A813	00	021
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Determination of Threshold Data From Coherent and Incoherent Radiation Sources

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

09600 Masers and Lasers; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 12	80 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	1.5	66
FISCAL YEAR CURRENT 81	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Eye Protection; (U) Infrared Lasers; (U) Systems Safety; (U) Laser Hazard; (U) Eye Damage; (U) Skin Damage

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objectives are to experimentally determine dose response relationships for infrared laser radiation for exposure conditions relevant to Army laser systems operation and to recommend permissible exposure limits based upon these bioeffects data.

24. (U) The ED_{50} (effective dose required to produce a specified response 50% of the time) for various exposure conditions and response criteria are determined. Cornea effects are evaluated at various time intervals by direct observation, histological techniques, and specular microscopy on Rhesus monkey eyes.

25. (U) 7910-8010. Corneal dose-response relationships were determined for infrared laser wavelengths. These results and exposure conditions are summarized as follows: 1) Neodymium laser at 1.064 μm, exposure duration 5 s, effective irradiance diameter 1.84 mm, ED_{50} = 825 J/cm^2, dose range tested 429-905 J/cm^2. 2) Neodymium laser operating at 1.318 μm and 1.338 μm simultaneously (38% of total energy at 1.318 μm and 62% at 1.338 μm), effective irradiance diameter 1.4 mm, exposure duration 5 s, ED_{50} = 212 J/cm^2, dose range tested 64-430 J/cm^2; and effective irradiance diameter of 0.4 mm, exposure duration of 250 μs, ED_{50} = 45 J/cm^2, dose range tested 20-126 J/cm^2. 3) Corneal ED_{50}s were determined using a prototype CO_2 laser designator operating at 10.6 μm. The pulse repetition frequency was 10 Hz and ED_{50}s were determined for 1, 2, 5, 10, 100, and 1000 pulses with a corneal irradiance diameter of approximately 0.5 mm. The duration of each pulse in the train was 100 ns. The dependence of the ED_{50} (radiant exposure per pulse on the number of pulses) approximated $kn^{-\frac{1}{4}}$ where n is the number of pulses in the train. Both the dose required to produce a minimal corneal lesion and the depth of the response exhibit a wavelength dependence which are indicative of and correlate with the relative absorption properties of the cornea. This work unit has been incorporated into Agency Accession Number DA EO 6308.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3E162772A813 — Health Effects of Military Lasers

WORK UNIT NO. 021 — Determination of Threshold Data from Coherent and Incoherent Radiation Sources

The following investigation has been conducted under this work unit:

STUDY NO. 1 Ocular and skin effects of infrared laser radiation

Corneal dose-response relationships were determined for infrared laser wavelength. The ED_{50}s (the effective dose required to produce a corneal lesion, as observed with the slit lamp biomicroscope, 50% of the time) and exposure conditions are summarized as follows: 1) neodymium laser at 1.064 μm, exposure duration 5 s, effective irradiance diameter 1.84 mm, ED_{50} = 825 J/cm^2; 2) neodymium laser operating at 1.318 and 1.338 μm simultaneously (38% of the total energy at 1.318 μm and 62% at 1.338 μm), effective irradiance diameter 1.4 mm, exposure duration 5 s, ED_{50} = 212 J/cm^2, dose range 64-430 J/cm^2; and effective irradiance diameter of 0.4 mm, exposure duration of 250 μs, ED_{50} = 45 J/cm^2, dose range 20-126 J/cm^2. Corneal ED_{50}s were determined by using a prototype CO_2 laser designator operating at 10.6 μm. The pulse repetition frequency was 10 Hz and ED_{50}s were determined for 1, 2, 5, 10, 100, and 1000 pulses with a corneal irradiance diameter of approximately 0.5 mm. The duration of each pulse in the train was 100 ns. The dependence of the ED_{50} (radiant exposure per pulse) on the number of pulses in the train (N) approximated the $KN^{-1/4}$ where K is a constant. Both the dose required to produce a minimal corneal lesion and the depth of the response exhibit a wavelength dependence. These are indicative of and correlate with the relative absorption properties of the cornea.

The eyes of two Rhesus monkeys were exposed to a large field of diffuse argon laser radiation for 2-hour periods with a screen radiance of 10-12 X 10^{-6} W/cm^{2}sr. A total of 26 hours of exposure was accumulated over a 3-week period in the eyes of one animal and 40 hours in the other animal. Retinal tissue from the first animal is being evaluated at Western Ontario University under USAMRDC contract DAMD 17-80-G-9466. Tissue from the second monkey has been analyzed in this laboratory and by the Pacific Medical Center under USAMRDC contract DAMD 17-79-C-09132. Observed morphological alterations could not be conclusively linked to the laser exposure.

BODY OF REPORT

WORK UNIT NO. 021 — Determination of Threshold Data from Coherent and Incoherent Radiation Sources

STUDY NO. 1 — Ocular and skin effects of infrared laser radiation

PROBLEM

Current and proposed military laser systems operate in the infrared region of the electromagnetic spectrum beyond 1.4 μm. In the spectral region from 1.0 to 3.0 μm, the absorption coefficients of the outer ocular media (cornea, aqueous, lens, and vitreous) vary over three orders of magnitude. Although limited data are available for specific exposure conditons, the wavelength dependence of the dose-response relationships relevant to Army systems has not been adequately defined. Permissible exposure limits have been defined in TB MED 279; however, bioeffects data for exposure conditions in this spectral region may warrant change in permissible exposure limits and impact on the design and employment of military systems. With the development of CO_2 laser designators, bioeffects data are required for infrared wavelengths for pulse repetition rate conditions.

In previous work, Rhesus monkey spectral sensitivity for fine resolution criteria was permanently altered after repeated low-level exposure to diffuse argon laser radiation. These exposure conditions are comparable to those anticipated in the use of laser scanned visual displays. Research has continued both in this laboratory and under USAMRDC contracts to determine if a morphological correlate to the functional alteration is apparent by light and electron microscopic evaluation.

RESULTS AND DISCUSSION OF RESULTS

Corneal dose-response relationships were determined for infrared laser wavelengths to evaluate the wavelength dependence of the minimal corneal response. ED_{50}s (effective dose required to produce a corneal lesion, as observed with the slit lamp biomicroscope, 50% of the time) and the exposure conditions are summarized in Table 1. All exposures were placed in Rhesus monkey eyes and the ED_{50}s were determined by probit techniques.

Corneal lesions produced by a focused beam from a neodymium laser operating at 1.064 μm involved 1/2 to 3/4 of the corneal thickness. Lesions observed at one hour in the 210-220 J/cm^2 dose range

(exposure duration 5 s) were not visible one week after exposure. Lenticular opacities of the posterior pole were also evident for these exposure conditions.

TABLE 1

Wavelength (μm)	Exposure Duration (s)	Effective Irradiance Diameter (mm)	Dose Range Tested (J/cm^2)	ED_{50} (J/cm^2)
1.064	5	1.84	425-905	825
1.318, 1.338	5	1.4	64-430	212
1.318, 1.338	250 X 10^{-6}	0.40	20-126	45

Five-second exposure from the unfocused beam from the 1.3 μm continuous wave neodymium laser (38% of the total energy at 1.318 μm and 62% at 1.338) resulted in the production of corneal lesions and involved approximately 1/2 the corneal thickness. Lesions observed near the ED_{50} dose were not observed one week after the exposure with the slit lamp. No retinal or lenticular changes were observed for the dose range tested. The ocular response produced by the 1.3 μm pulsed neodymium laser (Table 1) involved the full corneal thickness at doses near the ED_{50}. The "track" or scar through the full corneal thickness was slightly tapered and was observed 3 months after exposure. At the higher doses, the lesion diameter increased, the opacity was more dense, and the scar throughout the entire corneal thickness was wider and more distinct.

A prototype CO_2 laser designator, received from the US Army Night Vision and Electo-Optic Laboratory and modified in this laboratory, was used to evaluate the corneal effects of repeated exposure at a pulse repetition frequency of 10 Hz. The duration of each individual pulse in the train was 100 ns at the 1/2 power points and the pulse reached extinction at 2 μs. The corneal ED_{50}s were determined for a beam diameter of approximately 0.5 mm for pulse trains that contained 1, 2, 5, 10, 100, and 1000 pulses. The preliminary results are given in Table 2. Analysis of spatial intensity distribution and the irradiance diameter may result in an adjustment of the final values. Lesions produced by 10.6 μm radiation at the ED_{50} dose only involved

the corneal epithelium and were not visible by slit lamp observation 48 hours after the exposure. At doses 2-3 times the ED_{50}, immediate ablation of the corneal epithelium resulted. All lesions produced stained with the topical application of sodium fluorescein. The dependence of the ED_{50} per pulse on the number of pulses in the train (N) approximated the $N^{-1/4}$ relationship observed in retinal exposures to repetitive pulses.

TABLE 2

Number of Pulses (N)	ED_{50} per pulse (J/cm^2)
1	0.65
2	0.61
5	0.41
10	0.33
100	0.27
1000	0.23

The eyes of two Rhesus monkeys were exposed to a large field of diffuse argon laser radiation for 2-hour periods with a screen radiance of 10-12 X 10^{-6} W/cm^2sr. A total of 26 hours of exposure was accumulated over a 3-week period in one animal and 40 hours in the other animal. Retinal tissues from the first animal are being evaluated under USAMRDC Contract DAMD 17-80-G-9466. Tissue from the retina of the second animal has been analyzed in this laboratory and by USAMRDC Contract DAMD 17-79-C-09132. A more extensive discussion of the results will be contained in the final reports from these two contracts. Both animals exposed during this reporting period had its right eye patched; this eye served as a control. The measured pupil diameter for the exposure conditions was 4 to 4.4 mm. Morphological alterations which were not considered artifactual were observed in the photoreceptors and the pigment epithelium. While changes were observed, few quantitative differences are presently discernible between the patched eye and the exposed eyes and consequently the alterations can not be conclusively linked to the repeated laser exposure.

Determination of Threshold Data From Coherent and Incoherent Radiation Sources (Cont)

CONCLUSIONS

The doses required to produce a biomicroscopically visible corneal lesion for laser radiation in the 1.0 to 3 μm region of the electromagnetic spectrum exhibit a wavelength dependence which correlates with the relative absorption properties of the cornea. The energy per pulse required to produce a threshold cornea lesion for 10.6 μm laser radiation at a pulse repetition frequency of 10 Hz decreases as the total number of pulses increases, thus indicating an additive effect.

RECOMMENDATIONS

Although additional experimental data are needed for long exposure durations and larger corneal irradiance diameter for infrared laser exposures from 1.4 to 3.0 μm, a generalized wavelength correction to current permissible exposures appears necessary based upon the relative absorption properties of the ocular media. Additional corneal damage threshold determinations at the wavelength of 1.732 μm are needed for the following reasons: 1) an erbium laser system is being developed to operate at that wavelength for use in "eye safe" training devices and 2) the absorption of the outer ocular media for wavelengths greater than 1.4 μm reaches an approximate minimum at this wavelength.

Permissible exposure limits for repetitive pulse conditions should be evaluated and revised to reflect trends indicated by the experimental data. Additional pulse repetition data are required for infrared wavelength. The dependence of the corneal ED_{50} on the corneal irradiance diameter should be determined for short and long exposure durations.

Evaluation of the morphological changes from repeated low-level exposure to diffuse argon laser radiation must be completed and coordinated with the contractual efforts.

PUBLICATIONS

1. STUCK, B.E., D.J. LUND, and E.S. BEATRICE. Ocular effects of laser radiation from 1.06 to 2.06 micrometers. In: Proceedings of the Society for Photo-Optical Instrumentation Engineers (Washington, DC, 7 April 1980), Vol 229, Ocular Effects of Non-Ionizing Radiation. pp 115-120

Determination of Threshold Data From Coherent and Incoherent Radiation Sources (Cont)

2. STUCK, B.E., D.J. LUND, and E.S. BEATRICE. Ocular effects of holmium (2.06 micrometers) and erbium (1.54 micrometers) laser radiation. Health Physics (in press)

3. STUCK, B.E., G. DE VILLEZ, E.S. BEATRICE, and H. ZWICK. Microscopic evaluation of Rhesus retina after repeated low-level exposure to diffuse argon laser radiation. (Abstract) Invest Ophthalmol 19:189, 1980

PRESENTATIONS

1. STUCK, B.E., D.J. LUND, and E.S. BEATRICE. Ocular effects of laser radiation from 1.06 to 2.06 micrometers. Presented at the Society for Photo-Optical Instrumentation Engineers (Washington, DC, 7 April 1980)

2. STUCK, B.E., G. DE VILLEZ, E.S. BEATRICE, and H. ZWICK. Microscopic evaluation of Rhesus monkey retina after repeated low-level exposure to diffuse argon laser radiation. Presented at the Association for Research in Vision and Ophthalmology (Orlando, Florida, May 1980)

3. STUCK, B.E., and D.J. LUND. The laser hazard and the status of laser protective materials. Presented to the Army Science Board (Letterman Army Institute of Research , Presidio of San Francisco, California, 23 September 1980)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OE 6308	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A878	BA	161 APC EL06
b. CONTRIBUTING	62772A	3E162772A813	00	022
c. CONTRIBUTING	STOG	80-7,2:4		

11. TITLE (Procede with Security Classification Code)*

(U) System Developer Assistance Studies in Laser Bioeffects

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

009600 Masers and Lasers; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 07	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	3.7	158
	CURRENT 81	12.1	512

19. RESPONSIBLE DOD ORGANIZATION

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NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Erbium; (U) Repetitive Pulse; (U) Ocular Hazard; (U) Laser Safety; (U) GaAs; (U) Neodymium

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) To provide bioeffect data base for safety documentation of laser training devices and to improve the accuracy of safety standards as applied to laser training devices. To evaluate the ocular hazard of near infrared lasers considered for future laser training devices.

24. (U) Determine ED_{50} versus number of pulses for repetitive pulse laser ocular exposure. Determine the ED_{50} for near infrared lasers.

25. (U) 7910-8009. Ocular damage thresholds were determined in Rhesus monkey for exposure to repetitive pulse neodymium (1064 nm) and erbium (850 nm) lasers. The ED_{50}s (energy/pulse) for 20 ns pulse duration Nd exposures at a PRF of 10 Hz were: 1 pulse, 99 μJ; 10 pulses, 39 μJ; 100 pulses, 34 μJ; 1000 pulses, 18 μJ; 10,000 pulses, 4.3 μJ. The ED_{50}s (energy/pulse) for 180 ns pulse duration Nd exposures at a PRF of 1000 Hz were: 1 pulse, 136 μJ; 2 pulses, 80 μJ; 3 pulses, 51 μJ; 6 pulses, 55 μJ; 70 pulses, 16 μJ, 1000 pulses, 10 μJ; 10,000 pulses, 11 μJ; 100,000 pulses, 3.3 μJ. The ED_{50}s (energy/pulse) for 180 ns pulse duration Er exposures at a PRF of 10 Hz were: 1 pulse, 12 μJ; 10 pulses, 5.9 μJ; 100 pulses, 2.7 μJ. The ED_{50} for retinal alteration in Rhesus monkey for exposure to a 1.33 μ neodymium laser was determined to be 355 mJ for a 650 μs pulse duration.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 88 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3E162772A813 Health Effects of Military Lasers

WORK UNIT NO. 022 System Developer Assistance Studies in Laser Bioeffects

The following investigation has been conducted under this work unit:

STUDY NO. 1 Project MILES

Retinal dose response relationships were determined for ocular exposure in Rhesus monkey to repetitive pulse neodymium laser (1064 nm) and erbium laser (850 nm) irradiation. The results are tabulated. N is the number of pulses per exposure, t is the duration of each pulse, and T is the duration of the exposure. The ED_{50} is expressed as μJ per pulse.

NEODYMIUM LASER - 1064 nm
PRF - 10 Hz t - 20 ns

T	N	ED_{50} (μJ/pulse)
20 ns	1	99
1 s	10	39
10 s	100	34
100 s	1000	18
1000 s	10000	4.3

NEODYMIUM LASER - 1064 nm
PRF - 1000 Hz t - 180 ns

T	N	ED_{50} (μJ/pulse)
180 ns	1	136
2 ms	2	80
3 ms	3	51
6 ms	6	55
74 ms	74	16
1 s	1000	10
10 s	10000	11
100 s	100000	3.3

ERBIUM LASER - 850 nm
PRF - 10 Hz t - 180 ns

T	N	ED_{50} (μJ/pulse)
180 ns	1	12
1 s	10	5.9
10 s	100	2.7
100 s	1000	1.2

These data all follow the relationship $ED_{50} = KN^{-1/4}$.

The ED_{50} for retinal damage in Rhesus monkey eye for 1330 nm neodymium laser irradiation has been determined. When the pulse duration is 650 μs, the retinal ED_{50} is 355 mJ total intraocular energy.

BODY OF REPORT

WORK UNIT NO. 022 System Developer Assistance Studies in Laser Bioeffects

STUDY NO. 1 Project MILES

PROBLEM

Military training devices using laser transmitters are widely deployed within the Army. Use of these devices exposes personnel to laser radiation. It is essential that the ocular hazard of lasers used in these training devices be completely understood, and that lasers presenting the minimum hazard be incorporated where feasible.

RESULTS AND DISCUSSION OF RESULTS

Dose response data have been obtained for exposure to repetitive pulse trains ranging in duration from 20 ns (single pulse) to 1000 s for two neodymium laser systems and one erbium laser system. The first neodymium laser emitted 20 ns duration pulses at a pulse repetition frequency (PRF) of 10 Hz. The second neodymium laser emitted 180 ns duration pulses at a PRF of 1000 Hz. The wavelength of these lasers was 1064 nm. The erbium laser emitted 180 ns pulses at a PRF of 10 Hz. The wavelength was 850 nm. The 10 Hz lasers were flashlamp pumped, pockel cell Q-switched devices. The 1000 Hz laser was a continuously pumped, acousto-optically Q-switched device. A dichroic beamsplitter having high reflectivity at the laser wavelength and high visual transmittance directed the laser beam into the eye of the monkey while permitting continuous viewing of the exposure site on the ocular fundus via fundus camera. The mirror and fundus camera were aligned so that the laser beam passed through the center of the ocular pupil and coincided with the crosshairs at the retina, thus facilitating selection and observation of the exposure site. A constant proportion of the beam energy was diverted into a reference detector for dosimetry. The energy at this detector was correlated with the energy entering the eye by placing a calibrated EG&G 580 radiometer at the eye position and determining the ratio of the energy received by the two detectors. The exposure duration was controlled by an electronic shutter. Neutral density filters were used to attenuate the beam energy to the desired exposure level.

The animals used in these experiments were Rhesus monkeys. The animals were anesthetized and the pupils dilated. For exposures of 10 s or longer, the eyes were immobilized by a retrobulbar injection of lidocaine. The eye was held open by a lid speculum during

exposure, and corneal clarity was maintained by periodic irrigation with normal saline.

For exposure durations of 100 s and less, 25 to 36 exposures were placed in a rectangular array in the extramacular retina, including one row of marker burns for subsequent location. Only four exposures of 1000 pulses per exposure were attempted at any one session because of difficulty in maintaining corneal clarity. The exposure sites were examined via ophthalmoscope one hour after exposure. The criterion for retinal damage was the observation of a lesion at this examination. The data were evaluated by probit analysis to determine the ED_{50} for each exposure condition.

The ED_{50} and associated 95% confidence limits were determined for pulse repetition frequencies of 10 Hz and 1000 Hz and for exposure durations from single pulse to 1000 s. These data are presented in Tables 1, 2, and 3. In these tables, the following definitions apply:

PRF = pulse repetition frequency

t = duration of each pulse in the train

T = total exposure duration

N = number of pulses per exposure $N = PRF \times T$

ED_{50} = ED_{50} expressed as total energy per exposure

ED_{50}/pulse = ED_{50} expressed as energy per pulse

$ED_{50}/pulse = ED_{50}/N$

95% limits = 95% confidence limits for the ED_{50}/pulse

TABLE 1
Neodymium laser - wavelength 1064 nm
PRF = 10 Hz t = 20 ns

T	N	ED_{50} (μJ)	ED_{50}/pulse (μJ)	95% limits (μJ)
20 ns	1	99	99	83-120
1 s	10	389	39	32-47
10 s	100	3410	34	29-41
100 s	1000	18300	18	17-20
1000 s	10000	42800	4.3	2.9-6.4

TABLE 2
Neodymium laser - wavelength 1064 nm
PRF = 1000 Hz t = 180 ns

T	N	ED_{50} (μJ)	ED_{50}/pulse (μJ)	95% limits (μJ)
180 ns	1	136	136	107-173
2 ms	2	160	80	67-95
3 ms	3	153	51	40-65
6 ms	6	330	55	46-66
74 ms	74	1213	16	13-21
1 s	1000	10100	10	8.3-12
10 s	10000	115000	11	9.5-14
100 s	100000	330000	3.3	2.4-4.4

TABLE 3
Erbium laser - wavelength 850 nm
PRF = 10 Hz t = 180 ns

T	N	ED_{50} (μJ)	ED_{50}/pulse (μJ)	95% limits (μJ)
180 ns	1	12	12	9.5-15.1
1 s	10	59	5.9	4.7-7.5
10 s	100	270	2.7	2.0-3.5
100 s	1000	1200	1.2	.8-1.7

We gathered from the literature all available ocular damage threshold data for repetitive pulse exposures. From these data, an empirical relationship was derived which equated the ED_{50}/pulse in a pulse train to the ED_{50} for a single pulse and the number of pulses in the pulse train. The relationship

$$ED_{50}/\text{pulse} = KN^{-1/4}$$

where K is the ED_{50} for a single pulse of duration t, is valid for all of the repetitive pulse data examined. However, no data existed for large N, that is for long exposure durations. The experiment reported herein extended the data base to include data for long exposures and large N. It is evident that the empirical relationship continues to be valid for T = 1000 s and N = 100000 pulses. This result strengthens our recommendation that the provisions of the safety standards be modified accordingly.

A neodymium laser was modified by replacement of the resonator mirrors to operate in the 1300 nm wavelength band. The output energy consisted of 40% 1313 nm and 60% 1338 nm radiation. The pulse duration was 650 microseconds. At these wavelengths, optical absorption in the ocular tissue provides significant protection against retinal damage; by modifying the exposure parameters the primary damage site can be shifted to either the cornea or the retina. The corneal ED_{50} was determined in the last reporting period to be 45 J/cm^2. In this reporting period, the corneal beam diameter was increased so that the corneal irradiance was below the damage threshold in order to determine the retinal damage threshold. The procedures were as described above. The ED_{50} for retinal damage in Rhesus monkey was determined to be 355 mJ total intraocular energy.

The 1300 nm neodymium laser presents a relatively high ocular safety margin. The corneal damage threshold is a factor of three greater than the damage threshold for the 1540 nm erbium laser. The retinal damage threshold is a factor of 1000 greater than the damage threshold of the 1064 nm neodymium laser.

CONCLUSIONS

Repetitive pulse lasers pose a significantly greater ocular hazard than do continuous wave or single pulse lasers. The energy per pulse required to produce ocular damage decreases as the fourth root of the number of pulses. This relationship continues to hold for long exposure durations and large numbers of pulses. The neodymium laser operating in the 1330 nm wavelength band presents a significantly reduced ocular hazard when compared to lasers operating in the visible spectrum or to the 1060 nm neodymium laser.

RECOMMENDATIONS

It is recommended that the provisions of the Army laser safety standards as applied to repetitively pulsed lasers be changed to reflect more accurately the damage threshold data by setting C_p equal to $N^{-1/4}$. It is also recommended that the 1330 nm neodymium laser be strongly considered for use in laser training devices where personnel safety is a consideration.

PUBLICATIONS

None

REPORTS

1. LUND, D.J., B.E. STUCK, and P.A. O'MARA. Quarterly Scientific Progress Review; Laser Hazard Technology. Prepared for Commander, U.S. Army Medical Research and Development Command, Fort Detrick, Maryland, 16 June 1980

2. LUND, D.J. Quarterly Scientific Progress Review; Project MILES - Laser Technology. Prepared for PM TRADE, Orlando, Florida, 13 August 1980

3. LUND, D.J., B.E. STUCK, and P.A. O'MARA. Quarterly Scientific Progress Review; Laser Hazard Technology. Prepared for Commander, U.S. Army Medical Research and Development Command, Fort Detrick, Maryland, 16 October 1980

System Developer Assistance Studies in Laser Bioeffects (Cont)

PRESENTATIONS

LUND, D.J., and E.S. BEATRICE. Bioeffects Research in Support of Project MILES. Presented at Project MILES in Progress Review, Orlando, Florida, November 1979

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6102	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	B. Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3A162772A813	00	023 APC 5045
b. CONTRIBUTING	61102B	3E161102BS01	00	203
c. CONTRIBUTING				

11. TITLE (Procode with Security Classification Code)

(U) Military Stress and Combat Effectiveness

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

013400 Psychology; 005900 Environmental Biology; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 08		DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	2.4	90
FISCAL YEAR CURRENT 81	0.0	0

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME: Stamper, D.A., DAC

NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procode EACH with Security Classification Code)

(U) Military Performance; (U) Human Performance; (U) Visual Tracking; (U) Psychological

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procode text of each with Security Classification Code.)

23. (U) The severe stress encountered in warfare may influence the soldier's ability to perform combat-essential activities with maximum efficiency. The objectives of this research are to study 1) weapons effects on the performance of military tasks, 2) weapons systems environments and combat effectiveness, and 3) biomedical factors limiting soldier effectiveness. Research is conducted under field conditions and in the laboratory.

24. (U) Animals or human subjects are subjected to conditions which produce stress of varying intensity and durations. The effects of stressors are confirmed biochemically and through observation of physiological and psychological indices. Experimental stress is then related to the ability of subjects to perform various tasks. For human subjects, target acquisition and tracking, communications, endurance, and vigilance tasks are employed. Operant techniques are used with animal subjects.

25. (U) 7910-8010. Trained volunteers used a viscous-damped mount optical tracking system during a study of the effects of strobe flashes and ambient lighting on pursuit tracking performance. Targets moved to the left or right at an angular velocity of 5 mrad/s. Single, 170 μs, 538 nm strobe flashes produced significant increases in aiming errors. Recovery times of approximately 2 s were observed following flashes presented under bright ambient light conditions. Under low ambient light conditions, recovery times greater than 6 s were observed. The effects of spot size, retinal location, wavelength, and evasive target maneuvers will be examined during future studies. This work unit has been incorporated into Agency Accession Number DA OE 6308.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A162772A813 Health Effects of Military Lasers

WORK UNIT NO. 023 Military Stress and Combat Effectiveness

The following investigation has been conducted under this work unit:

STUDY NO. 6 Biomedical factors affecting laser-designator operator performance

EX-2 Countermeasures directed against laser-designator operators; high intensity quasi-monochromatic flashes

Ten, trained, male volunteers used a viscous-damped mounted optical tracking system during a study of the effects of strobe flashes and ambient lighting on pursuit tracking performance. The volunteers tracked a 0.5 mrad target moving to the left or right for 15 s at a constant angular velocity of 5.0 mrad/s. A single 170 μs, 0.053 sr, 538 nm strobe flash was presented at random at the rate of one flash for each 5 trials. The flashes produced significant increases in the standard deviations of the horizontal and vertical aiming errors under both ambient light conditions. The average maximum aiming error was 0.6 mrad during bright ambient light trials. Approximately 2 s were required to return to normal control error rates. Flashes presented during the low ambient lighting conditions produced off-scale errors (>2 mrad). Recovery times averaged 6 s for a 1.0 mrad target and 3 s for a 4.0 mrad target. This study used single large retinal area strobe flashes (0.053 sr) that were an order of magnitude below permissible safe exposure levels and much lower than levels produced by military laser devices. The flashes produced significant disruptions of pursuit tracking performance even though the behavior of the target was predictable. It is not known whether the same effects would be obtained when using smaller areas of retinal illumination, different wavelengths, or multiple flashes.

BODY OF REPORT

WORK UNIT NO.	023	Military Stress and Combat Effectiveness
STUDY NO.	6	Biomedical factors affecting laser-designator operator performance
EX-2		Countermeasures directed against laser designator operators; high intensity quasi-monochromatic flashes

PROBLEM

Soldiers engaged in visual tasks may be exposed to high intensity light which could disrupt the performance of those tasks. Short duration wide spectrum photic stimulation may be produced by pyrotechnics, nuclear weapons, high intensity search lights, and electronic strobes. Lasers represent a significant new light hazard in the combat environment. Directed laser light in the visible spectrum may produce flash blindness or retinal damage. Infrared high energy laser radiation may vaporize the surfaces of optical devices. The resulting reradiation from the optical surface will also produce flash effects.

RESULTS AND DISCUSSION OF RESULTS

Ten, trained, male volunteers used a viscous-damped mounted optical tracking system during a study of the effects of strobe flashes and ambient lighting on pursuit tracking performance. The volunteers tracked a 0.5 mrad target moving to the left or right for 15 s at a constant angular velocity of 5:0 mrad/s. A single 170 μs, 0.053 sr, 538 nm strobe flash was presented at random at the rate of one flash for each five trials. The flashes produced significant increases in the standard deviations of the horizontal and vertical aiming errors under both ambient light conditions. The average maximum aiming error was 0.6 mrad during bright ambient light trials. Approximately 2 s were required to return to normal control error rates. Flashes presented during the low ambient lighting conditions produced off-scale errors (>2 mrad). Recovery times averaged 6 s for a 1.0 mrad target and 3 s for a 4.0 mrad target. This study used single large retinal area strobe flashes (0.053 sr) that were an order of magnitude below permissible safe exposure levels and much lower than levels produced by military laser devices. The flashes produced significant disruptions of pursuit tracking performance even though behavior of the target was predictable. It is not known whether the

same effects would be obtained when using smaller areas of retinal illumination, different wavelengths, or multiple flashes.

CONCLUSIONS

This study used single large retinal area strobe flashes (0.053 sr) that were an order of magnitude below permissible safe exposure levels and much lower than levels produced by military laser devices. The flashes produced significant disruptions of pursuit tracking performance even though behavior of the target was predictable. It is not known whether the same effects would be obtained when using smaller areas of retinal illumination, different wavelengths, or multiple flashes.

RECOMMENDATIONS

The effects of multiple, small retinal spot, chromatic or white flashes should be investigated.

PUBLICATIONS

O'MARA, P.A., D.A. STAMPER, D.J. LUND, and E.S. BEATRICE. Chromatic Strobe Flash Disruption of Pursuit Tracking Performance. Report No. 88. San Francisco, California: Letterman Army Institute of Research (Submitted for review and clearance)

PRESENTATIONS

O'MARA, P.A. Project BLASER: Flash countermeasure results. Presented at the Combat Ocular Problems and Night Vision Symposium at LAIR (San Francisco, California, October 1980)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA CE 6103	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AE	083 APC EL08
b. CONTRIBUTING	62772A	3E162772A813	00	024
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)*

(U) Care of the Combat-Injured Eye

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 05	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	3.6	100
FISCAL YEAR CURRENT 81	6.4	216

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Zwick, H., DAC POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Intraocular Trauma; (U) Laser Bioeffects; (U) Dark Adaptometer; (U) Ocular Physiology

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) (a) Perforating wounds of the posterior segment of the eye remain a difficult treatment problem. Methods to improve treatment of these injuries will be studied. (b) Laser devices are being used with increasing frequency by the Army. Since the effect of chronic low-level exposure is still unknown, studies to evaluate this potential hazard will be conducted. (c) Sufficient biological variation in dark vision exists to warrant the development of an instrument to separate individuals who have superior dark vision ability from those who have lesser ability.

24. (U) (a) The use of urokinase will be investigated in the rapid absorption of vitreal hemorrhage produced by laser radiation. (b) The optical behavior and pathological effects of low-level gallium arsenide laser will be studied in Rhesus monkey eyes in which a posterior window has been created to better observe these effects. (c) A dark adaptometer will be developed that can be used for screening.

25. (U) 7910-8009. (a) Urokinase, injected intravitreally, reduces the rate of complications in rabbits' eyes in which vitreous hemorrhage was produced by laser irradiation. (b) Several preparations of a posterior window in an enucleated Rhesus monkey eye have been done and photographs and other studies of a laser beam in the eye carried out. (c) The computer used to control the dark adaptometer has been replaced with a single board microprocessor.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3E162772A813 Health Effects of Military Lasers

WORK UNIT NO. 024 Care of the Combat-Injured Eye

The following investigation has been conducted under this work unit:

STUDY NO. 4 Treatment of corneal, retinal, and vitreal effects of laser injury

EX-1 Use of urokinase in rapid absorption of vitreal hemorrhage

Urokinase, injected intravitreally, reduces the rate of complications in rabbits' eyes in which vitreous hemorrhage was produced by laser irradiation.

BODY OF REPORT

WORK UNIT NO.	024	Care of the Combat-Injured Eye
STUDY NO.	4	Treatment of corneal, retinal, and vitreal effects of laser injury
EX-1		Use of urokinase in rapid absorption of vitreal hemorrhage

PROBLEM

Laser-induced vitreous hemorrhage will be common in the future battlefield. No acceptable clinical method exists to treat this condition except for vitrectomy, which is a hazardous operation and is performed only at a few specially equipped centers with qualified personnel. We attempted to use the fibrinolytic enzyme urokinase intravitreally to accelerate vitreous hemorrhage absorption.

RESULTS AND DISCUSSION OF RESULTS

Vitreous hemorrhages were produced in both eyes of six rabbits. One eye was injected with urokinase and the other with saline, two immediately, two at one hour, and two at 24 hours after injury. The best results were obtained in the rabbits which were injected 24 hours after injury. Six more rabbits underwent similar procedures and all were injected 24 hours after injury. The average test eye cleared three days before the control eyes cleared. This result is not clinically significant, none of the test eyes had any serious complication of the injury while all the control eyes had either vitreous fibrosis, or cataract, or both.

CONCLUSIONS

Urokinase accelerates absorption of laser-induced vitreous hemorrhage to a certain extent and prevents serious complication after that injury.

RECOMMENDATIONS

Tests in rabbits should be continued in efforts to verify results, refine time schedules, and to determine accurate dosage. Similar experiments should be performed on monkeys.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OE 6078	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CF	245 APC EL09
b. [redacted]	62772A	3A162772A813	00	025
c. [redacted]	STOG	80-7.2:4		

11. TITLE (Precede with Security Classification Code)*

(U) Biological Investigations in Prediction and Protection Against Coherent Radiation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

009600 Masers and Lasers; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 12	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	4.5	380
	CURRENT 81	11.0	493

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Randolph, D.I., DAC POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Laser Systems Safety; (U) Laser Hazard; (U) Eye Damage; (U) Human Visual Test Battery

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective is to determine the effects of safe exposure laser radiation levels, as determined largely by gross morphological criteria, on the visual processes of non-human primates (Rhesus monkeys) and other lower animal species of less expense but of equal relevance.

24. (U) Behavioral and neurophysiological procedures are used to measure visual function. Low-level acute or chronic exposure conditions are established for each experiment based on known morphological experimental results or present laser safety standards. Measures of visual acuity, spectral sensitivity, dynamic acuity, visual neuronal, and ultrastructural morphology are used as measures and procedures.

25. (U) 791 -8009. 1. Repeated very low-level exposure (100 times or more below Maximum Permissible Exposure levels (MPE)) have produced changes in acuity and spectral sensitivity that have not recovered for more than three years. Neurophysiological correlates in non-human primates and vertebrates have provided evidence that basic neuronal visual processes can be altered by such low levels of laser radiation. In many cases, effects have been delayed or have required repeated exposure. These kinds of effects have not been considered thus far in the development of laser or other standards for non-ionizing radiation sources primarily in the visible and near infrared regions of the spectrum. 2. Effects of brief (20 ns) minimal spot PRF laser exposure has shown that transient changes in visual acuity and contrast threshold can be produced many times below burn threshold. Permanent changes in color vision processes were obtained at MPE levels for single 100 ms exposures. 3. Progress was made in designing a vision test battery incorporating dark adaptation, contrast sensitivity, spectral sensitivity, and acuity for static and moving targets.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A162772A813 Health Effects of Military Lasers

WORK UNIT NO: 025 Biological Investigations in Prediction and Protection Against Coherent Radiation

The following investigation has been conducted under this work unit:

STUDY NO. 1 Effects of laser irradiation on visual function

Repeated very low-level exposure of visible diffuse (514 nm) laser light produced changes in Rhesus acuity and spectral sensitivity that lasted for more than three years after the exposures. The levels of diffuse irradiation employed were several hundred times below maximal permissible exposure levels for extended sources, well below levels that produce visible retinal lesions. Two Rhesus monkeys have been trained and tested so far in these experiments. Parallel neurophysiological and morphological investigations in both Rhesus monkeys and lower vertebrates strongly suggest that neural coding within the visual system is altered by such exposure. In preliminary investigations, the transient and residual effects of pulsed point source visible laser (532 nm) exposure have been investigated. These preliminary data indicate that point source pulsed laser exposure at 532 nm can produce significant transient losses in maximal visual acuity as well as changes in the contrast sensitivity function at levels near or below the retinal burn threshold. Present investigations are concerned with both lower level exposure effects on transient as well as residual effects on visual function.

BODY OF REPORT

WORK UNIT NO.	025	Biological Investigations in Prediction and Protection Against Coherent Radiation
STUDY NO.	1	Effects of laser irradiation on visual function

PROBLEM

The major problem in this research is the determination of laser radiation effects on vision that may result from accidental exposure, as well as from levels of exposure that may be used in new laser training systems, and possible effects that may result from tactical situations. Animal subjects, primarily Rhesus monkeys, are used because their visual system and behavior are similar to man's. Other lower vertebrates are used as animal models to study in detail low-level damage mechanisms of coherent light exposure.

RESULTS and DISCUSSION OF RESULTS

Two Rhesus monkeys were exposed to diffuse levels of argon laser radiation (514 nm) at screen radiance of 6 $\mu W/cm^2 sr$ which corresponds to a retinal irradiance of 0.2 $\mu W/cm^2$ over the entire retina in binocular view. Two-hour daily exposures cumulating to a total dose of 38 hours of exposure were given to each animal. Both animals showed significant losses in sensitivity for the very small gap sizes. These effects were not immediate but occurred only after a total of 10 to 20 hours of exposure had been accumulated in each animal. Subsequent follow-up examinations in both animals showed no recovery of these losses in spectral sensitivity. Initial losses in spectral sensitivity appeared at first to be specific to a single class of cone photoreceptors but follow-up studies in both animals over more than three years indicate that effects were permanent and also appeared to progress long after termination of exposure.

Three additional animals were tested on spectral sensitivity with electroretinogram (ERG) as the criterion response. Low-level voltage analysis using a Lock-In Amplifier frequency analysis servolooped for a voltage criterion of 0.5 μV rms was employed. These animals were exposed to higher levels of 514 nm laser radiation in Maxwellian view, but the levels were still below the maximum permissible exposure for the extended source criterion. Losses in photopic spectral sensitivity corresponding with an increase in rod photoreceptor system dominance were obtained. These effects are presently being followed.

Biological Investigations in Prediction and Protection Against Coherent Radiation (Cont)

Several experiments were done in the cone-rich retina of Pseudemys to determine the neural mechanisms underlying low-level laser damage effects. In the first experiment, ERG spectral sensitivity using a Lock-In Amplifier servoloop system was employed to determine if the effects of laser light might be different from incoherent light or time-averaged laser light for equivalent retinal irradiances and peak wavelengths. Data obtained in this experiment indicated that the "speckle" pattern produced by laser light at a surface can be a relevant factor in producing the effects observed in Pseudemys as well as in Rhesus. Similar experiments in which neuronal activity of optic tectal neurons of Pseudemys were measured also indicated that laser "speckle" could be the relevant factor in producing low-level laser effects. Exposures at or below those made in Rhesus behavioral experiments produced permanent disruption of measured optic tectal neuronal activity. At these levels, cumulative effects were always apparent and combining multiple laser wavelengths in the visible region produced obvious enhancements of these deleterious effects.

A technique for assessing rapidly the effects of long pulse laser exposure (100 ms) was developed by using visual evoked potentials to alternating grating stimuli. Assessment of transient laser flash effects is possible with this technique as is measurement of low-level residual effects on macular visual function. Low-level 60 second GaAs laser radiation effects upon the visual system were observed and documented.

Brief repetitive flash exposure (20 ns, 10-20 pulses per second) for visible (532 nm) point source laser light produced significant transient changes in Rhesus visual acuity and contrast sensitivity at exposure levels at or slightly below those levels capable of producing retinal burn. Transient deficits of similar magnitude were also obtainable at exposure levels 100 times below our maximum exposure levels. Single Q-switched pulses (20 ns) produced transient effects that were generally delayed by several minutes whereas multiple pulses produced immediate large deficits in acuity. The ability of the visual system to respond to a brief flash may involve different neurochemical processes in the nanosecond time domain.

CONCLUSIONS

Coherent light can produce prolonged changes in vision that appear related to the unique stimulation that laser light produces on the retinal surface. Such effects have been found many orders of magnitude below the present maximum permissible exposure limits. The effects appear to be of neural coding origin rather than of direct morphological involvement. These low-level coherent light experiments

Biological Investigations in Prediction and Protection Against Coherent Radiation (Cont)

are still in progress. Both the initial sample size and the mechanism of alteration are receiving intensive study. Brief point source light when pulsed can produce transient and severe losses in visual acuity and contrast sensitivity. These investigations are being extended to explore lower level exposure flashes as well as the possibility of residual effects.

RECOMMENDATIONS

- Additional animals should be trained and tested on the behavioral tasks so far employed. Two animals have been prepared this year for these follow-up studies

- Parallel studies should be conducted on Rhesus by using electrophysiological criteria such as ERG and Visual Evoked Potential.

- Attempts to defeat laser "speckle" should be devised for Rhesus behavioral experiments.

- Morphological parallel studies in naive animals should be continued and expanded as warrented by data.

- Human visual function tests should be devised to test humans rapidly and efficiently who may have to be exposed to chronic laser radiation at levels otherwise deemed safe by present laser permissible exposure guidelines. Some of these tests have been developed in the Division of Biorheology under a separate protocol. Collaborative work with the Army Environmental Hygiene Agency and several outside Army contractors involved in Laser Training Display systems has been ongoing.

- New concepts in optical protection for low-level laser hazards must be explored. A laser dosimeter (badge) which would measure the amount of cumulative weekly exposure is highly recommended.

PUBLICATIONS

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Biological Investigations in Prediction and Protection Against Coherent Radiation (Cont)

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3. ZWICK, H., B.E. STUCK, and E.S. BEATRICE. Low-level laser light effects - long-term effects. In: Proceedings of the 24th Annual Meeting of the Human Factors Society (Los Angeles, California, 1980). pp 152-156

4. ROBBINS, D.O., H. ZWICK, and M. HAENLEIN. Changes in spectral acuity following laser irradiation. In: Proceedings of the 24th Annual Meeting of the Human Factors Society (Los Angeles, California, 1980). pp 162-166

5. ZWICK, H., P.A. O'MARA, E.S. BEATRICE, S.L. BIGGS, and C.W. VAN SICE. A solid-state dark adaptometer - the LAIR dark adaptometer. In: Proceedings of the NATO/AGARD Specialists Meeting (Toronto, Canada, 1980) (In press)

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9. BLOOM, K.R., and ZWICK, H. Rhesus spectral dynamic visual acuity. (Abstract) In: Proceedings of Recent Advances in Vision Topical Meeting of the Optical Society (Sarasota, Florida, 1980). p WA 3

Biological Investigations in Prediction and Protection Against Coherent Radiation (Cont)

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12. ROBBINS, D.O, H. ZWICK, and M. HAENLEIN. Changes in spectral acuity following laser irradiation. (Abstract) In: Supplement to Invest Ophthalmol Visual Sci, April 1980 (for Annual Spring Meeting, ARVO, Orlando, Florida, 4-9 May 1980). p 91

13. STUCK, B.E., G. DE VILLEZ, E.S. BEATRICE, and H. ZWICK. Microscopic evaluation of Rhesus retina after repeated low-level exposure to diffuse Argon laser radiation. (Abstract) In: Supplement to Invest Ophthalmol Visual Sci, April 1980 (for Annual Spring Meeting, ARVO, Orlando, Florida, 4-9 May 1980). p 189

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 3372	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 01 17	D. Change	U	U		NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	093 APC HL04
b. [redacted]	62772A	3S162772A814	00	003
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)

(U) Laser Acceleration of Soft Tissue Wound Healing

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 008800 Life Support; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 01	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	0.5	14
	CURRENT 81	1.2	38

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Belkin, Michael

NAME: Beatrice, Edwin S., COL, MC

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Wound Healing; (U) Military Trauma; (U) Animal Model; (U) Laser

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Experimental data exist suggesting that laser irradiation of full-thickness skin defects accelerates wound healing. It is necessary to confirm these findings and to determine whether or not the extent to which wounds heal faster is clinically significant.

24. (U) Full thickness skin defects of a standard size will be created in rabbits. One group will be treated by daily dressing changes, while a second group will also be irradiated every third day with a helium neon laser. Wound surface area will be measured every day and statistical comparison made between the control and treated groups. Further studies will include measurement of tensile strength in excised-sutured wounds with and without laser exposure.

25. (U) 80 01 - 80 09 Laser irradiation of full thickness skin defects does not alter the rate at which full thickness wounds close or the rate at which tensile strength increases in healing wounds. We intend to repeat these experiments using a 50 mw laser (ten times prior power level--energy delivered to wound will remain 2 erg/cm^2). Work will also start using the full thickness model to assess the reported benefit of using amniotic membrane as a wound dressing.

*Available to contractors upon originator's approval.

DD FORM 1498 (1 MAR 68)

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 003 Laser Acceleration of Soft Tissue Wound Healing

Laser irradiation accelerates the healing of soft tissue wounds, according to reports in the European literature. A rabbit model has been developed at LAIR to investigate the effect on healing of a 5 mW helium-neon laser delivering 2 J/cm^2 every third day to a standard wound. No difference has been demonstrated in absolute wound area, rate of healing, or tensile strength between laser-irradiated and untreated control wounds. The experiment will be repeated with the use of a laser that will deliver 10 times the power of the previous study.

BODY OF REPORT

WORK UNIT NO. 003 Laser Acceleration of Soft Tissue Wound Healing

PROBLEM

The Hungarian surgeon Janos Meister has reported that laser irradiation accelerates healing in a variety of wounds including chronic decubiti in humans and freshly sutured incisions in rats (Panminetva Medica 17:229, 1975; Experientia 30:1296, 1974). The mechanism by which the laser affects healing remains uncertain but may involve more rapid formation of cross-links between collagen fibrils secondary to an increased concentration of superoxide radicals in the irradiated tissue. Finding ways of increasing the rate at which full thickness tissue defects heal have military relevance because such wounds are common following debridement of high velocity through-and-through gunshot wounds. We have attempted to reproduce certain portions of Dr. Meister's work. Full thickness skin defects on the back of rabbits were irradiated with a 5 mW helium-neon laser, 2 J/cm^2 of energy being delivered to the wound every third day during a 30-minute exposure period. Wound surface area was measured daily and compared with an untreated control. Following healing, wounds were excised and the force per unit area required to cause disruption was measured. Wound tensile strength has also been measured in sutured incisions in rats, one group serving as a control and a second group being irradiated with a laser.

RESULTS AND DISCUSSION OF RESULTS

We have been unable to demonstrate any difference in absolute wound area, rate of healing, or tensile strength between laser irradiated and untreated wounds. We have discussed our results with Dr. Meister, who suggests that the experiments be repeated with a 50 mW laser applied daily rather than every third day and that the eschar covering the wound not be disturbed.

CONCLUSIONS

We have been unable to show that laser irradiation of soft tissue wounds accelerates wound healing.

RECOMMENDATIONS

The effect of laser irradiation upon wound healing will be restudied by using Dr. Meister's most recent recommendations.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOE 6087	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. Change	U	U		NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AD	084 JL03
b. [redacted]	62772A	3S162772A814	00	004
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)

(U) CPDA-2 Clinical Trials

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 01	CONT	DA	C. IN-HOUSE

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	1.9	55
FISCAL YEAR CURRENT 81	1.5	67

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Bolin, Robert B.,LTC , MC POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Blood Storage; (U) Military Blood Banking; (U) Red Cell Survivals; (U) Adenine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) The final objective of this study, clinical trials of an improved anticoaglant is Food and Drug Administration's licensure, which would permit clinical use of red cells after prolonged liquid storage. Shipment of blood into combat areas necessitates delays between drawing and infusion; the impact of these delays on the quality of red cells infused will be minimized through use of an improved anticoagulant-preservative solution.

24. (U) Currently, red cell liquid storage in CPDA-1 anticoagulant-preservative is limited to 35 days. Survivability of packed red cells (PC) stored in CPDA-1 for 35 days is marginally acceptable. In vitro studies of metabolism in red cells and platelets stored in modified CPD-adenine suggest that increased adenine and glucose in the preservative will improve survivability. Such improvements may allow extension of red cell storage time to 42 days or beyond. The Division of Blood Research, LAIR, is coordinating efforts with civilian and container-solution manufacturers in the execution of clinical trials of promising improved CPD-adenine formulations and CPDA-2.

25. (U) Human in vivo red cell survival studies are currently underway in an effort to extend blood storage to 42-56 days. Completed studies indicate that the survivability of packed red cells and whole blood stored in CPDA-2 for 35 days is significantly superior to that of blood stored in CPDA-1. Furthermore, erythrocyte viability is well preserved after 42 days of storage. Preliminary results of studies performed at 49 and 56 days suggest that viability may be preserved in CPDA-2 for prolonged periods of storage.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 004 CPDA-2 Clinical Trials

The following investigations have been conducted under this work unit:

STUDY NO. 1 Platelet studies - CPDA-2

STUDY NO. 2 Red cell studies - CPDA-2

STUDY NOS. 1 and 2. Recent collaborative efforts with several laboratories, which were initiated and guided by the Division of Blood Research, LAIR, culminated in the development and FDA approval of a new preservative, CPDA-1. CPDA-1 was a marked improvement over CPD and ACD. It extended shelflife by 67% and improved the quality of preserved red cells. CPDA-1 preservative is not optimal and in vitro studies suggest that improved formulations may extend storage beyond 35 days even for packed red cells. The preservative CPDA-2 has been selected as the best formulation of adenine and glucose. To establish human utility and to obtain FDA approval, clinical trials for both red cells and platelets are being performed. In vivo red cell survival studies, CPDA-2 is far superior to CPDA-1 at day 35 for preservation of whole blood and packed cells (hematocrit = 75%). In addition, studies completed at 42 days of storage have indicated that CPDA-2 fulfills the FDA criteria for approval of a new blood preservative solution. Preliminary results from 49 and 56 day studies indicated that CPDA-2 may be used for the preservation of whole blood and packed cells for extended storage periods. After 8 hours preprocessing delay, platelets removed from whole blood are stored for 72 hours and then transfused into the original donor. CPDA-2 stored platelets are compared to control (CPD) platelets. Preliminary results suggest CPDA-2 is as good as CPD for platelet storage.

BODY OF REPORT

WORK UNIT NO. 004 CPDA-2 Clinical Trials

STUDY NO. 1 Platelet studies CPDA-2

PROBLEM

Before FDA approval of a new preservative, it must be documented that the solution will not adversely affect any usable component of blood such as plasma proteins and platelets. Approval of CPDA-1 was delayed due to the lack of data concerning the effect of the preservative on platelets. CPDA-2, the new preservative developed in part by the Division of Blood Research to optimize the concentrations of adenine and glucose for red cell storage, is now ready for clinical trials. Concurrent with red cell storage, platelet studies should be performed to insure the preservative is not injurious to this blood component.

RESULTS AND DISCUSSION OF RESULTS

Normal volunteers have been used to obtain in vivo data about platelets prepared and preserved in CPDA-2. Studies are currently in progress with conventional storage but using an 8-hour hold after phlebotomy before processing. Results are being generated for CPDA-2 and a control preservative, CPD. To date, 4 CPDA-2 and 4 CPD blood samples have been evaluated (total of 6 each to be studied). CPDA-2 is not inferior to CPD in these studies as determined by platelet harvest, pH of the stored product, in vivo recovery and survival.

CONCLUSIONS

The data, when complete, should provide documentation for the Bureau of Biologics to consider if CPDA-2 is an acceptable preservative for platelets after they have been held in an 8-hour preprocessing mode.

RECOMMENDATIONS

These studies should be completed as soon as possible. If 8-hour preprocessing hold is detrimental to platelet storage, further studies should be developed to meet Federal requirements to insure CPDA-2 approval. Approval of this preservative will greatly enhance blood logistic support for the military.

STUDY NO. 2 Red cell studies CPDA-2

PROBLEM

Recent collaborative efforts by several laboratories, which were initiated and guided by the Division of Blood Research, LAIR, culminated in the

development and FDA approval of a new preservative, CPDA-1. This preservative was a marked improvement over CPD and ACD. It extended shelflife from 21 to 35 days and improved the quality of red cells. CPDA-1 is not an optimal preservative, particularly for packed cells stored for 35 days. Studies in this laboratory suggest that adjustments in the concentration of adenine and additional glucose could result in greater than 35-day storage and an improvement in the quality of stored packed cells. This approach has marked impact for military needs since prolonging the shelflife of blood will improve logistic support for combat zone needs. The better the preservative, the more universal its acceptance. The use of a military-oriented preservative by civilian blood banks will insure military needs are met from existing blood supplies.

RESULTS AND DISCUSSION OF RESULTS

Intramural studies to evaluate red cell survival rates in whole blood and packed red cell units (each at 35, 42, 49, and 56 days of storage) and to determine the maximum acceptable length of storage have been initiated. The results of studies performed at 35 and 42 days are summarized below:

CPDA-2 RED CELL SURVIVAL
(24-Hour Post-Transfusion Survival)

	Whole Blood (%)	Packed Red Cells (%)
35 days storage	84.4±5.58 (N=5)	91.5±5.90 (N=4)
42 days storage	74.4±9.95 (N=5) (one below 70%; i.e., 57.7%)	76.2±6.94 (N=9) (two below 70%; i.e., 66.9% and 68.1%)

In vitro biochemical studies performed on these units indicated changes associated with blood storage that are comparable to CPD and CPDA-1. In addition, preliminary studies have been performed to evaluate the efficacy of CPDA-2 as a blood preservative solution for whole blood and packed red cell units stored for 49 and 56 days. These studies indicate that this preservative fulfills FDA criteria for at least 49 days of storage (24-hour red cell survival: whole blood, 49 days, 76.7% [N=1], packed cells, 49 days, 74.6±1.3% [N=2]; whole blood, 56 days, 65.1±8.7% [N=4]; packed cells, 56 days, 69.2% [N=1]). These results have been corroborated by extramural studies performed by Dr. E. Buetler, La Jolla, CA.

CPDA-2 Clinical Trials

CONCLUSIONS

These studies have established that CPDA-2 is capable of successfully preserving whole blood and red cells for 35 and 42 days of in vitro storage. Preliminary results suggest that the maximum storage capacity may be extended to 49 or 56 days.

RECOMMENDATIONS

In vivo red cell survival studies should be completed for 49 and 56 days of storage in CPDA-2 in FY 81.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 2389	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	248 APC HL07
b. [redacted]	62772A	3S162772A814	00	010
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)*

(U) Investigating a Circulating Shock Factor of Pancreatic Origin

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002300 Biochemistry; 012600 Pharmacology; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
79 10	CONT	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	2.8	141
	CURRENT 81	5.1	227

19. RESPONSIBLE DOD ORGANIZATION

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NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Pancreas; (U) Vascular Monitoring; (U) Kinin; (U) Shock; (U) Pancreatic Shock Factor

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) The pancreas is known to contain substances that produce shock. We have found a shock factor that is produced only when minced pancreas is exposed to collagenase. We wish to determine how this shock factor affects blood pressure and flow. We also wish to characterize the chemical properties, isolate and purify, and block the effects of this agent.

24. (U) We will monitor the blood vessel effects of this shock factor in a dog and pig model. This response will be our standard to compare physical and chemical manipulations of the agent. Pharmacologic agents will be tested for blocking capability.

25. (U) 79 10 - 80 09 Our pancreatic shock factor (PSF) significantly decreased total systemic resistance and increased portal venous resistance from baseline values. This response was duplicated by bradykinin but not endotoxin or trypsin. An agent isolated from the dog salivary gland, but not other canine tissues, produces a similar hypotensive agent. We hope to investigate further the kinin system and how it might be activated by PSF.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 010 Investigating a Circulating Shock Factor of Pancreatic Origin

The following investigations have been conducted under this work unit:

STUDY NO. 1 Hemodynamic characterization of a pancreatic shock factor (PSF)

STUDY NO. 2 Isolation and purification of PSF

STUDY NO. 1. The pancreas is known to contain substances that produce shock, i.e., a persistent decrease of blood pressure and flow. Hemorrhagic shock has been found in combat-injured soldiers where resuscitative measures have been delayed. We have isolated a pancreatic shock factor (PSF) which is obtained by collagenase digestion of minced pancreas. PSF was hemodynamically characterized in 32 mongrel dogs by measuring femoral artery pressure (FAP), portal pressure (PP), central venous pressure (CVP), pulmonary artery pressure (PAP), left atrial pressure (LAP), ascending aortic flow, i.e., cardiac output (CO), and portal venous flow (PoVF). Vascular resistance was then calculated for each vascular bed. Injections of PSF were made into the FAP, CVP, PAP, and PP catheters. Other organs (muscle, lung, submandibular gland, liver, kidney, stomach antrum, duodenum, and ileum) were processed similarly to the pancreas and tested for hemodynamic activity. Also tested were known hypotensive agents: bradykinin, trypsin, and endotoxin. PSF, injected at any of the sites, significantly decreased total systemic resistance, significantly increased portal venous resistance, and had no effect on total pulmonary resistance. CO increased as FAP decreased. The submandibular gland was the only other organ tested which possessed this vascular activity. A vascular response which mimicked the PSF vascular response occurred when bradykinin was administered. Trypsin and endotoxin lowered FAP but depressed CO. The data suggest that glandular kallikrein, present in both pancreas and submandibular gland, activates the kinin system to vasodilate the peripheral vascular bed with secondary effects of increasing CO and decreasing PoVF.

STUDY NO. 2. When minced canine pancreas is digested with collagenase, an agent is liberated in the soluble fraction which will cause shock in minute doses. Each of the components of the digestion (collagenase, Hank's balanced salt solution, or minced pancreatic tissue) will not produce shock by itself. The pancreatic shock factor (PSF) of Study No. 1 is contained in the supernatant of collagenase-digested canine minced pancreas. We wished to isolate and purify PSF by lyophilization to concentrate the supernatant. The concentrate was applied to a series

of 6 chromatography columns packed with differing types of Sephadex G. Each gel allowed a progressively larger molecular weight substance to be present in the void volume. This initial fraction was then tested for PSF activity in a canine in vivo bioassay. Several experiments have been carried out and the preliminary results indicate that PSF is probably a macromolecule greater than 30,000 daltons. However, the upper limit has not yet been determined.

BODY OF REPORT

WORK UNIT NO.	010	Investigating a Circulating Shock Factor of Pancreatic Origin
STUDY NO.	1	Hemodynamic characterization of a pancreatic shock factor (PSF)

PROBLEM

The low blood pressure associated with shock may progress to an irreversible stage if not treated. Any delay of resuscitative measures in a combat-injured soldier may lead to irreversible shock. The pancreas releases shock-inducing substances during periods of low blood pressure and may promote an irreversible condition. We have isolated a substance from the pancreas which causes shock when placed in the vascular system. This pancreatic shock factor (PSF) is obtained by exposing minced pancreas to collagenase. Our objective is to determine the typical vascular response to a shock factor derived from the pancreas, ascertain if the pancreas is the only tissue containing a shock factor, investigate the mechanism of action (dose-response curves, mimetic agents, pharmacologic blockade), determine the thermal characteristics, and determine if the components of the digestion process (minced pancreas, collagenase, or balanced salt solution) were capable of inducing a vascular response.

RESULTS AND DISCUSSION OF RESULTS

The typical vascular response was determined following a bolus injection of a supernatant from a canine pancreas. The gland had been excised, minced, and digested with collagenase. This pancreatic shock factor (PSF) caused a statistically significant decrease in total peripheral resistance ($p<0.01$) and an increase in portal venous resistance ($p<0.01$). No significant change was noted in pulmonary artery resistance. This overall vascular response to PSF occurred regardless of the injection site, i.e., portal vein, right atrium, left atrium, or femoral artery. The time for onset of reaction was shortest if the PSF was infused just proximal to or directly into the peripheral vascular tree. The main site of action was therefore probably in the systemic vasculature. Confirmatory evidence was found when the total peripheral resistance was first to change as compared to resistances in the portal, venous, or pulmonary systems.

Multiple organs and tissues (muscle, lung, liver, kidney, stomach, duodenum, ileum, and submandibular gland) were processed similar to the pancreas and tested by injection into the portal vein. Only the submandibular gland produced a vascular response and this reaction was the same as the response to PSF. Both the pancreas and the submandibular gland contain high concentrations of glandular kallikrein, an activator of the kinin system. Bradykinin, obtained commercially, when injected

into dogs produced a similar vascular response as the PSF or the supernatant of the submandibular gland. This suggested that the kallikrein-kinin system may be involved. Other agents that lower total peripheral resistance, trypsin and endotoxin, were tested but they depressed cardiac output as well. Since activators of the kinin system are required in small doses, a dose-response curve to PSF was obtained which showed that minute doses of PSF (0.002 cc/kg) were necessary to result in a vascular response which varied from baseline values by a statistically significant margin ($p<0.05$). The PSF response occurred in the simian and porcine models as well as the canine. A kinin blocker, aprotinin, was injected along with the PSF in each of these three animal species. In the monkey, the vascular response to PSF was unaffected by aprotinin (even with 10,000 KIU/kg). In the dog, the elevation of portal pressure was blocked by aprotinin, but not the fall in blood pressure. In the pig, both elevated portal pressure and fall in blood pressure were blocked by aprotinin.

Thermal characteristics of PSF were tested by freezing and heating for 30 minutes to 60 C, 80 C, or 100 C. Boiling was necessary to inactivate the PSF when injected into the canine portal vein. Freezing PSF did not alter the vascular response. The components of the digestion process (minced pancreas, collagenase, or balanced salt solution) did not produce a vascular response.

CONCLUSIONS

The pancreas contains glandular kallikrein which in minute doses may activate the kinin system to cause shock. The process is prevented in the pig by a kinin blocker and mimicked by commercial bradykinin. Species differences exist for the effectiveness of kinin blockade. The failure to prevent irreversible shock in primates may lie in the differences in species response to kinin blockade. The understanding of the pathophysiology of shock may possibly be answered by species-specific kallikrein-kinin blockade.

RECOMMENDATIONS

The kallikrein-kinin system should be investigated by using commercially obtained porcine kallikrein and determining its vascular response in the porcine model. A new kinin blocker developed in Japan (FOY) should be tested in all three animal models: pig, dog, and monkey. Other species sources of aprotinin should be sought (which is currently available only from bovine lung). The kinin system should be more precisely measured than by the crude in vivo bioassay. We are developing methods to measure kallikrein and kinin by using chromozyme PK and an in vitro bioassay and radioimmunoassay possibly with the aid of a hybridoma, respectively. The serum kallikrein-kinin system should be measured in animals during PSF-induced shock.

Investigating a Circulating Shock Factor of Pancreatic Origin (Cont)

PUBLICATIONS

1. TRAVERSO, L.W., and R.R. GOMEZ. Investigation of a circulating shock factor obtained from canine pancreatic autografts. (Abstract) Proceedings of the Annual Meeting of the Pancreas Club, American Gastroenterological Association Week, Salt Lake City, Utah, April 1980.

STUDY NO. 2 Isolation and purification of PSF

PROBLEM

A pancreatic shock factor (PSF) is present in the supernatant resulting from centrifugation of collagenase (bacterial enzyme) digested minced pancreatic tissue. The objective of this study is to isolate, purify, and characterize canine PSF.

RESULTS AND DISCUSSION OF RESULTS

PSF is measured by an in vivo assay which is detailed in Study No. 1. The source of PSF has been the supernatant from collagenase-digested canine pancreatic minced tissue. Earlier attempts to isolate PSF by thin membrane ultrafiltration yielded inconclusive results since PSF activity was present in both filtrate and retentate of different molecular-sized membranes. PSF activity was also present in all fractions of Sephadex G100 and G10 gel columns in which sodium azide (NaN_3) was used as an antimicrobial agent in buffer solution. A 0.02% solution of sodium azide caused an in vivo PSF-like response.

Recent purification methods no longer utilize sodium azide. In the current method we use small chromatographic columns (7 mm in diameter), each packed with a different Sephadex G gel which has a different molecular range over which molecules of different sizes can be fractionated. Materials of known molecular weights (e.g., blue dextran, phenol red, bovine serum albumin, etc.) are used to calibrate the columns. Lyophilization is used to concentrate the PSF before application to columns. PSF in vivo-vascular activity is present in the exclusion volume of G10, G15, G25, and G50 columns, which indicates a molecular weight greater than 30,000 daltons. Results with G100 and G200 are distorted, probably because of the viscosity of the concentrates.

CONCLUSIONS

An antimicrobial agent used in column chromatography buffers, sodium azide, causes a PSF-like response using the in vivo assay detailed in Study No. 1. Eliminating sodium azide from buffer solutions and the use of Sephadex G gel column chromatography techniques enabled the separation of PSF activity into a molecular range greater than 30,000 daltons. PSF could also consist of multiple chemical agents which are not eluted in the same fraction.

Investigating a Circulating Shock Factor of Pancreatic Origin (Cont)

RECOMMENDATIONS

PSF should be identified and its role in irreversible shock should be studied. Large scale preparation of PSF should be carried out from pancreas obtained from a slaughterhouse. Chromatographic column fractions should be analyzed with a sensitive in vitro assay system such as an in vitro bioassay or radioimmunoassay for kinin.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE 6077	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AB	086 APC HL09
b. [redacted]	62772A	3S162772A814	00	012
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

008800 Life Support; 016200 Stress Physiology; 009800 Medical and Hospital Equipment

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 11	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	0.2	13
	CURRENT 81	4.5	106

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U)Combat Surgery;(U)Trauma;(U)Wet-Lung Syndrome;(U)Left Ventricular Function;(U)Oxyhemoglobin Disc;(U)Artificial Blood;(U)Combat Anesthesia

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Newly developed artificial blood substitutes and whole blood stored using new techniques must be physiologically evaluated to insure their ability to support tissue function. The objective of this work unit is to develop and use an appropriate subprimate model which will permit precise measurements of ventricular hemodynamic and metabolic function. This model has been used to investigate the importance of different anesthetic agents under conditions of combat stress, and to test the effectiveness of newly developed blood substitutes and blood having altered oxyhemoglobin dissociation characteristics.

24. (U) A perfused in situ swine heart model using total and right heart bypass with control of heart rate, blood pressure, and left heart loading pressure has been employed. Left heart performance and myocardial oxygen transport dynamics will be assessed to determine the ability of newly developed blood substitutes and anesthetic agents to support normal tissue function under combat stress.

25. (U) 79 10 - 80 09 A cardiovascular investigational laboratory is functioning for active measurements of stroke volume, dp/dt, injection fraction, myocardial metabolism, and coronary flow distribution. Animals subjected both to anemia and perfusion with blood having an altered oxygen-hemoglobin affinity have shown no change in function with increased affinity, but have shown a lowered coronary sinus and myocardial PO_2 value. Stroma-free hemoglobin solution perfusions to a hematocrit of 5% in this model have resulted in sustained heart performance, whereas albumin perfusion to a hematocrit of 5 or 10% has not allowed sustained heart function. Current studies are examining stroma-free hemoglobin solution perfusion at higher hematocrit levels. Anesthetic agents are being examined under conditions of combat field care with reference to the efficiency of heart work.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 012 Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier

The following investigations have been conducted under this work unit:

STUDY NO. 2 The effect of variation in the oxyhemoglobin dissociation curve on left ventricular function in swine

STUDY NO. 3 Anesthetic agents and their effect on left ventricular function during normoxia and hypoxia

STUDY NO. 4 The effect of stroma-free hemoglobin solution on myocardial function in a nonshock, subtotal exchange model

STUDY NO. 2. The relationships between preservation of myocardial performance and the oxyhemoglobin dissociation curve of priming solutions have been investigated in the isolated swine heart preparation described previously. These studies have been designed to determine whether or not the P_{50} of resuscitation fluids, including whole blood, is a significant determinant of recovery from hemorrhagic shock secondary to massive combat wounds. Animal studies have been completed indicating that variations in P_{50} have a significant effect on left ventricular function at normal oxygen tensions and hemoglobin concentrations. Further studies examining the role of P_{50} variation during anemia have been evaluated and preliminary analysis shows that anemia does not heighten the effects of a change in P_{50} on left ventricular function, but increased affinity does result in decreased coronary sinus PO_2 values and decreased myocardial tissue PO_2 values. Further work is underway to determine whether or not the oxyhemoglobin dissociation curve has an important part in determining myocardial performance during hypoxia and limited coronary artery blood flow.

STUDY NO. 3. The myocardial effects of the major anesthetic agents have been studied in our swine heart model in an attempt to evaluate these agents under conditions analogous to combat-induced stress. Previous studies completed under this work unit have substantiated that halothane decreases left ventricular function during normoxia and especially during hypoxia, whereas morphine sulfate had a minimal effect. Further analysis of this depression has revealed that the decrease in function was due more to a decrease in myocardial compliance rather than a decrease in contractility. Further investigations are being conducted to define this effect of anesthetic agents during situations of combat stress.

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

STUDY NO. 4. Work has continued to progress in the evaluation of stroma-free hemoglobin solutions on myocardial performance. In initial studies with the in situ swine heart model, we evaluated a subtotal exchange transfusion comparing stroma-free crystalline hemoglobin solution with a 7% bovine albumin solution to produce a hematocrit level of 5%. These studies show that while myocardial performance was decreased by approximately 50% with stroma-free hemoglobin solution, the animals were able to maintain this level of cardiac performance; whereas, animals exchanged with the albumin solution were unable to sustain any degree of myocardial work. Animals with a hematocrit of 10% exchanged with albumin were also unable to sustain significant cardiac work. Studies are now in progress examining the potential value of stroma-free hemoglobin solution at hematocrit levels above 5%.

BODY OF REPORT

WORK UNIT NO. 012 — Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier

STUDY NO. 2 — The effect of variation in the oxyhemoglobin dissociation curve on left ventricular function in swine

PROBLEM

Recently, with the understanding that the oxyhemoglobin dissociation curve is affected by concentrations of 2,3-diphosphoglycerate (2,3-DPG) and that stored blood has a low 2,3-DPG level, there has been concern that massive transfusions with blood which has been stored for prolonged periods may have a detrimental effect on oxygen delivery to critical tissues. Myocardial function is intimately tied to adequate oxygen transport which, if less than optimal, may depress heart performance in the combat-injured soldier. Some studies have suggested that there is a relationship between P_{50} and left ventricular performance. If an adequate P_{50} is crucial to preserving heart performance during periods of combat injury, then aged blood with a low P_{50} and low 2,3-DPG may have limited usefulness, and fresh blood or blood with enriched 2,3-DPG must be made available. If P_{50} is not a major determinant of left ventricular function, aged blood could be employed, especially during combat situations which would require massive transfusions and maximal utilization of blood bank resources.

RESULTS AND DISCUSSION OF RESULTS

Our in situ perfused swine heart model has been used for this study. As previously outlined, left ventricular function and metabolic responses have been directly evaluated.

Currently, in this study, we are evaluating myocardial function following exchange transfusions with blood having various P_{50} characteristics and hematocrit levels. As reported previously, our initial study with this preparation examining the situation at a normal hematocrit level showed that the left ventricular performance is affected in an adverse fashion when animals are subjected to blood having a lowered P_{50}. This change in performance was accompanied by documented and statistically significant changes in the P_{50}, n-value, and coronary sinus gas values for the animals. The group of animals subjected to exchange with high P_{50} blood had preservation of myocardial performance but did not show an improved or superior performance to that with blood having a normal P_{50} value. A second phase of this study has examined the effect of altered P_{50} in the left ventricular function in an animal exchanged with blood at a lowered hematocrit level. These animal studies have

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

been completed. They revealed that increased oxygen-hemoglobin affinity during anemia does not result in decreased left ventricular function when compared to exchange transfusion with blood having a decreased oxyhemoglobin affinity. Increased affinity did result in a lowered tissue and coronary sinus PO_2 value. This finding indicates a lower level of oxygen availability in the tissues of the working myocardium being perfused with low P_{50} blood.

CONCLUSIONS

Our basic conclusion is that P_{50} is an important determinant of left ventricular function. The question of its clinical importance remains to be answered. Further studies will examine the situation in animals stressed at a lower hematocrit level, and in situations of decreased oxygen tension.

RECOMMENDATIONS

The findings in this study have helped to answer the question of how vital a role P_{50} changes play in myocardial performance. Additional work is needed to weigh adequately the role in a clinical situation analogous to that experienced by soldiers on the combat field. The problem of evaluating the role of P_{50} in myocardial performance is being assessed with an in situ swine model. We may also use a less expensive small animal model. We recommend 1) the publication of animal experiments performed under this work unit; and 2) continued work examining this question in other animal models. Some of this work will be accomplished in the Letterman Army Institute of Research facility. Much work will also be accomplished in extramural laboratories with which the principal investigator is presently associated.

PUBLICATIONS

None

STUDY NO. 3 Anesthetic agents and their effect on left ventricular function during normoxia and hypoxia

PROBLEM

The effects of anesthetic agents on myocardial function have been well worked out for the normal situation encountered in civilian operating room practice where the patient is at an optimum oxygenation level. Unfortunately, during combat situations patients may have to be anesthetized during conditions of decreased oxygen tension. The ultimate survival of these patients is closely connected with their myocardial performance. Safe anesthesia would require optimization of myocardial

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

performance even during conditions of hypoxia. This information becomes crucial if the field anesthesiologist is to select the optimal available anesthetic agent during these combat stress situations. In the past, this particular problem has been addressed in Work Unit No. 008, DAOE6305, Anesthetic Management and Perioperative Care of the Acutely Wounded Soldier. During the last year work has been conducted under Work Unit No. 012, DAOE6077 and is reported here.

RESULTS AND DISCUSSION OF RESULTS

The perfused swine heart model has been used and animal studies examining the response of halothane, morphine, and infiltration anesthetic regimens have been conducted. The technique for accurately measuring anesthetic concentrations with a mass spectrometer and the technique for accurately adjusting the animal's oxygen tension to a level of 40 torr have been perfected. With these technical refinements, it has been possible to complete the evaluation of a series of animals at normoxia and at the hypoxic level of 40 torr. The initial results from this study have shown that halothane, as expected, decreases myocardial performance during normoxia. This drop in performance is accompanied by a decrease in myocardial oxygen consumption. The new finding during hypoxia was that halothane anesthesia not only drops myocardial performance significantly more during hypoxia, but also that this drop in performance is not accompanied by a corresponding drop in oxygen consumption. The experiments performed with morphine anesthesia substantiated that, under conditions of normoxia, morphine has no appreciable depressive effects on myocardial performance and that its depressive effects during hypoxia are relatively less than with halothane (approximately 25% versus 66%). This depression in function is not accompanied by an increase in myocardial oxygen consumption. The results from the animals examined under infiltration anesthesia were similar to those with the animals examined under morphine anesthesia. These data have been analyzed in order to determine the mechanism for the change in myocardial performance seen with halothane.

CONCLUSIONS

Our basic conclusion is that halothane may be an appropriate anesthetic agent to use during normoxic conditions since the depression of myocardial performance is accompanied by a decrease in myocardial oxygen consumption. The amount of oxygen consumed per unit of cardiac work is not increased which prevents ischemic damage to the myocardium. During hypoxia, halothane is not a good anesthetic since the depression of myocardial function is enhanced and this depression is accompanied by an increased oxygen consumption, thereby subjecting the myocardium to a greater risk of ischemic damage.

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

RECOMMENDATIONS

Additional work is needed to examine the anesthetic agents during periods of hypoxia and other situations of deranged physiology such as hypotension and anemia that are encountered in a combat injury situation. The question of an appropriate choice of an anesthetic agent during situations of combat stress needs to be answered by additional studies examining various anesthetic agents during anemia and hypotension in the controlled swine heart model. Some technique modifications will probably be required to answer the question completely whether the depression in myocardial performance with halothane is due to a direct depression of myocardial contractility or due to a change in ventricular compliance. We will use ultrasonic crystals placed in the myocardium to measure myocardial dimensions directly during anesthetic administration.

PUBLICATIONS

1. MOORES, W.Y., R.B. WEISKOPF, M. BAYSINGER, and J.R. UTLEY. Effects of halothane and morphine sulfate on myocardial appliance following total cardiopulmonary bypass. J Thor Cardiovasc Surg (in press)

STUDY NO. 4 The effect of stroma-free hemoglobin solution on myocardial function in a nonshock, subtotal exchange model

PROBLEM

Resuscitation of the combat injured soldier may require the use of various artificial blbod substitutes as well as whole blood. These solutions must be adequately evaluated in terms of their effects on myocardial function. Several studies examining stroma-free hemoglobin solutions have been accomplished in a shock model. However, it is appropriate to examine the effects of these resuscitation techniques in an animal model which allows evaluation of myocardial function in a nonshock situation as might be encountered during recovery and convalescence from combat injury. This study should help determine if casualties should be transfused with hemoglobin solution or an artificial blood substitute that carries oxygen, or if a nonoxygen carrying blood substitute, such as albumin solution, would be adequate.

RESULTS AND DISCUSSION OF RESULTS

During the last year, the in situ perfused swine heart model has been used to evaluate the effects of an exchange transfusion of stroma-free hemoglobin solution on left ventricular function. The standard parameters of myocardial performance (stroke volume, etc.) have been examined under conditions of controlled pre-load, after-load, and rate, and an index of myocardial metabolism and oxygen utilization has been

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

used. These studies have been done with hemoglobin solution that has been exchanged in a pig animal model so that the subsequent hematocrit was 5%. Experiments comparing stroma-free hemoglobin solution with albumin solution to produce a hematocrit level of 5% has revealed that animals transfused with the stroma-free hemoglobin solution were able to maintain a work performance at approximately 50% of their control value and were able to sustain this level of work performance for the standard work trial period. The animals exchanged with the albumin solution to produce a similar hematocrit level were initially able to support the same level of cardiac performance; but, within minutes of the work trial period, these animals were no longer able to perform any useful cardiac work. Those animals perfused with stroma-free hemoglobin solution showed signs of inadequate oxygen delivery, such as, high lactate levels. However, the hearts were able to work with the stroma-free hemoglobin solution. Albumin exchanged to produce a hematocrit of 10% did not allow myocardial work to be sustained.

CONCLUSIONS

Stroma-free hemoglobin solution is promising in terms of supporting useful cardiac work under conditions of severe anemia. Support of cardiac function occurred even though the present form of hemoglobin solution has a depressed P_{50} with a left-shifted oxyhemoglobin dissociation curve.

RECOMMENDATIONS

Additional work is necessary to define the role of stroma-free hemoglobin in those situations where the hematocrit level is not severely depressed. Continued work should be done to improve the solution so that cardiac performance can be maintained without causing anaerobic metabolism. We are evaluating stroma-free hemoglobin solution perfusion at hematocrit levels that are greater than 5%. We are also evaluating improved solutions which have a more normal oxyhemoglobin dissociation curve and with better in vivo retention..

PUBLICATIONS

1. MOORES, W.Y., F. DEVENUTO, W.H. HEYDORN, R.B. WEISKOPF, M. BAYSINGER, and J.P. HANNON. Improved porcine myocardial performance during severe anemia using a stroma-free hemoglobin solution. (Abstract) Fed Proc 39:709, 1980
2. MOORES, W.Y., F. DEVENUTO, D.C. WILLFORD, and J.R. UTLEY. Stroma-free hemoglobin solution in a non-shock model. (Abstract) Proceedings, Current Concepts of Combat Casualty Resuscitation Symposium, Bethesda, Maryland, 1980

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

3. MOORES, W.Y., F. DEVENUTO, W.H. HEYDORN, R.B. WEISKOPF, M. BAYSINGER, and J.R. UTLEY. Extending the limits of hemodilution on cardiopulmonary bypass using stroma-free hemoglobin solution. J Thorac Cardiovasc Surg (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DA OE 6100	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	246 APC HL10
b. [redacted]	62772A	3S162772A814	00	013
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)*

(U) Effect of Blood -Oxygen Affinity During Experimental Hemorrhagic Shock and Hypoxemia

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 012900 Physiology; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	0.6	35
	CURRENT 81	2.0	75

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign Literature Reviewed

POC: DA

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Resuscitation Solutions; (U) Experimental Hemorrhagic Shock; (U) Trauma; (U) Blood-gas Transport; (U) Acetylcholinesterase

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) To evaluate relationships between gas transport functions of blood and compensatory physiologic responses to combat trauma, particularly that producing blood loss and cyanosis. To determine safe, effective and practical means of manipulating hemoglobin-oxygen affinity in cases where this would benefit the natural defenses of combat casualties against traumatic injury, chemical agents and disease.

24. (U) Animal models are used to simulate injury encountered in combat casualties, including hemorrhage, burns, fractures, blunt trauma, acetylcholinesterase inhibitors and other noxious agents. Physiologic parameters (heart rate, cardiac output, ventilation, oxygen consumption, regional blood flow, blood and tissue gases, pH, hemoglobin-oxygen affinity, hematocrit, viscosity, oncotic pressure and other pertinent variables) are measured and the status of oxygen transport to tissues evaluated. Hemoglobin-oxygen affinity is manipulated (administration of stored blood and blood substitutes, sodium cyanate, sodium bicarbonate, etc.) to observe the effects of such manipulation on morbidity and mortality.

25. (U) 79 10 - 80 09 Hemoglobin-oxygen affinity (P_{50}) was significantly associated with the response of rats to administration of the acetylcholinesterase (AChE) poison diisopropylfluorophosphate (DFP). Administering a lethal dose (4 mg/kg) of DFP to rats with normal P_{50} of approximately 40 mm Hg resulted in 75 and 100 percent mortality at six and 24 hours, respectively. Similar challenge of cyanate-treated rats with P_{50} of approximately 22mm Hg gave 32 ($P<.01$) and 75 ($P<.05$) percent mortality at similar times. Preliminary data indicates the latter group has improved arterial oxygenation, larger art.-ven. oxygen differences and less marked fall in body temperature. Providing a lower blood P_{50} apparently compensates in part for reduced ventilation following AChE inhibition, suggesting a supplemental means of treating chemical warfare casualties.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 013 Effect of Blood Oxygen Affinity during Experimental Hemorrhagic Shock and Hypoxemia

Hypoxemia and cyanosis are prominent early signs of anti-cholinesterase (anti-ChE) poisons and effective therapy requires adequate oxygenation with assisted breathing and supplemental oxygen as well as alleviation of persistent cholinergic hyperactivity with atropine and oximes. The proximate physiologic cause of hypoxemia with this form of chemical poisoning is inability to achieve adequate alveolar ventilation, leading to lowered alveolar oxygen tension and failure to saturate circulating hemoglobin. It was hypothesized that under these circumstances, oxygenation of blood would be improved and hypoxic sequelae alleviated in part by shifting the oxyhemoglobin dissociation curve to the left thus permitting more complete saturation of hemoglobin at reduced oxygen tensions. The appropriateness of this maneuver was tested by observing responses to diisopropylfluorophosphate (DFP) in normal rats and in rats treated with potassium cyanate, an agent that increases hemoglobin oxygen affinity. Preliminary results have revealed a significant association in rats between hemoglobin-oxygen affinity and the development of mortality and morbidity after DFP administration. The experiments provide evidence that increased hemoglobin-oxygen affinity compensates in part for reduced ventilation following ChE inhibition and suggest a supplemental means of treating organophosphorus-poisoned combat casualties.

BODY OF REPORT

WORK UNIT NO. 013

Effect of Blood-Oxygen Affinity during Experimental Hemorrhagic Shock and Hypoxemia

PROBLEM

Acute interruption of the normal oxygen supply to tissues is the most serious potential consequence of exposure to organophosphorus poisons. Case histories based on accidental exposure to cholinesterase (ChE) inhibitors consistently refer to respiratory distress and cyanosis, symptoms that graphically emphasize the need for maintaining oxygenation in the patient. It is somewhat surprising that bradycardia is not a prominent feature of the early course of ChE inhibition, given the expectation that acetylcholine accumulation would enhance the vagotonic effect on the heart. Apparently, the fulminating nature of the hypoxic stress in such cases can override cholinergic slowing of the myocardium.

Under ideal conditions permitting prompt definitive medical care, the requirement for improving oxygen transport to tissues after organophosphorus poisoning ordinarily is met by the judicious use of atropine, assisted respiration and supplemental oxygen. Oximes (2-PAMCl, for instance) are also applied as detoxifying agents while maintaining supportive care. The possible use of chemical agents during hostilities, however, would substantially complicate the conditions under which medical care could be provided in such cases. Massive numbers of casualties as well as the remoteness and difficulties of logistical support of combat zones would conspire to compromise the quality of medical treatment. Professional management, for instance, would be minimal and supportive care (assisted respiration, supplemental oxygen, etc) would be non-existent in most instances. Widespread indiscriminant self-adminstration of atropine, moreover, might produce more casualties than the chemical warfare agents themselves in some situations. For these reasons, the current research was designed to explore the pathophysiology of organophosphorus inhibitors, particularly in relation to gas transport phenomena. It was hypothesized that by shifting the hemoglobin-oxygen affinity to the left, a better match would be obtained between the oxygenation characteristics of hemoglobin and the existing oxygen tensions in pulmonary alveoli, thus avoiding an acute interruption of oxygen supply to tissues. Such chemical "impedance matching" is actually fairly commonplace in nature and a number of examples of favorable adaptive behavior by hemoglobin to low environmental oxygen have been reported.

RESULTS AND DISCUSSION OF RESULTS

Results, using an experimental rat model, have revealed a significant association between hemoglobin-oxygen affinity and the occurrence of mortality and morbidity after lethal doses of diisopropylfluorophosphate

Effect of Blood-Oxygen Affinity during Experimental Hemorrhagic Shock and Hypoxemia (Cont)

(DFP). DFP (4mg/kg) administrered subcutaneously to rats (N = 31) with normal hemoglobin-oxygen affinity (P_{50} = 40 mm Hg) resulted in accumulated mortality of 45,75 and 100% at 2, 6 and 24 hours respectively. A group of rats (N = 26) previously treated with potassium cyanate, which altered the P_{50} of hemoglobin to an average value of 22 mm Hg, displayed an accumulated mortality of 22, 32 and 75% at 2, 6 and 24 hours, respectively, after receiving 4 mg/kg DFP subcutaneously. By chi-square analysis, these differences were statistically significant at 6 and 24 hours ($P < 0.01$ and < 0.05, respectively). At two hours, the difference was not statistically significant although there were fewer deaths in the high affinity group.

Preliminary data indicate that rats having left-shifted oxyhemoglobin dissociation curves also have improved arterial hemoglobin saturation, larger arterial-venous oxygen differences as well as a less-marked fall in body temperature after receiving DFP. Potassium cyanate did not significantly alter plasma or erythrocyte ChE activity and the major acute effect of this material seems to be related exclusively to carbamylation of hemoglobin and increased hemoglobin-oxygen affinity. Hemoglobin levels in controls and cyanate treated animals were not remarkably different although there may be a slight tendency toward minor increases with prolonged cyanate treatment.

Data obtained so far support the initial hypothesis that increasing hemoglobin-oxygen affinity assists the organism in overcoming hypoxemia associated with acute organophosphorus poisoning and diminishes the likelihood of a fulminating cyanotic episode. At the lethal dose levels used, all the animals exposed to DFP eventually died, even though compared to controls, there was about a five-fold increase (2 hours to 10 hours) in the time required for half of the cyanate-DFP treated animals to succumb. It is not clear presently whether or not the apparent improvement in arterial oxygenation found in the cyanate-treated animals may have prevented hypoxic stimulation of the heart, thereby unmasking the expected vagotonic effect on this organ from ChE poisoning. If so, the resulting bradycardia with improved arterial hemoglobin saturation apparently compromised overall homeostasis less severely, at least immediately, than did the alternative conditions in the controls.

The interpretation of the influence of hemoglobin-oxygen affinity on the course of DFP poisoning in these experiments is complicated by the fact that the reported P_{50} values were obtained from *in vitro* measurements. In the face of inadequate respiratory compensation during DFP poisoning, continuing tissue hypoxia and anaerobic metabolism will gradually reduce blood pH, as noted in a number of acute preparations. Acidemia, by lowering hemoglobin-oxygen affinity through the *in vivo* Bohr shift, will accentuate any inability of hemoglobin to combine with oxygen in pulmonary capillaries. Thus, even though cyanate-treated rats cope

initially with the pulmonary insufficiency of organophosphorus poisoning more readily than control rats, oxygen transport is probably not fully restored by such treatment and localized oxygen deficits lead inexorably, albeit at a slower pace, to lower blood pH, diminished hemoglobin-oxygen affinity, and increased hypoxemia. This vicious cycle is apparently slowed but not broken by inducing a high initial hemoglobin-oxygen affinity. The inhibition of ChE at neuromuscular junctions is generally refractory to atropine. Atropine is most effective in diminishing excessive secretions and moderating bronchoconstrictor activity. Cyanosis consequently, may be present even after massive doses of atropine because breathing muscles, particularly the diaphragm, cannot adequately ventilate the lungs.

CONCLUSIONS

Increasing hemoglobin-oxygen affinity in rats with potassium cyanate favorably influences the pathophysiologic sequelae from DFP poisoning. Present data appear to support the hypothesis that this result is obtained through a better matching of hemoglobin-oxygen combining characteristics with existing (low) alveolar oxygen tensions. In effect, the physiologic pulmonary shunt is reduced without any apparent alteration in the ventilation/perfusion ratio, hypoxemia and cyanosis are reduced, and the organism is better able to mount an effective compensatory adjustment to ChE inhibition.

RECOMMENDATIONS

The effect of altered hemoglobin oxygen affinity on the outcome of DFP challenge should be measured in conjunction with the use of 2-PAMCl. Other means of altering hemoglobin-oxygen affinity should be investigated. Confirmatory experiments using a larger animal model should be performed.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE 6108	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	087 APC HL11
b. [redacted]	62772A	3S162772A814	00	015
c. [redacted]	STOG	80-7,2:5		

11. TITLE (Procede with Security Classification Code)*

(U) Animal Models for Surgical Repair of Musculoskeletal Structures

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology; 003500 Clinical Medicine; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 05	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	0.3	27
FISCAL YEAR CURRENT 81	0.3	17

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Surgical Repair; (U) Extensor Tendon; (U) Nerve; (U) Muscle Transplantation; (U) Trauma; (U) Nerve Graft; (U) Microsurgical Technique

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Extremity nerve injuries in military personnel are extremely costly. To minimize the resulting lost duty days, permanent disability, and the expenditure of medical resources, efforts are being made to improve current surgical and therapeutic techniques in order to return personnel to duty with maximum function in the minimum time.

24. (U) Segmental defects simulating combat injuries of 0, 1, 2, or 3 cm were created in both ulnar nerves in 16 cynamologus monkeys. These monkeys then underwent repair of the defects either epineurially with tension, or interfascicularly with sural nerve grafts. Critical evaluation of the neurorrhaphies was accomplished 5 months after the repairs. To determine the rate and morphometric pattern of axon regrowth across an anastomosis, ulnar nerves of 6 rhesus monkeys were severed, repaired, and then biopsied at either 1, 2, 3, 4, 5, or 6 week intervals.

25. (U) 79 10 - 80 09 There was no statistical difference between the 2 repair techniques in overcoming segmental nerve defects in cynamologus monkeys. The technical difficulty which was encountered with large defects justifies the use of sural nerve grafts in overcoming large defects. Light and electron microscopic evaluation showed that axon regrowth occurs immediately and that an anatomical recovery is not possible, regardless of the surgical technique.

*Available to contractors upon originator's approval.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 015 Animal Models for Surgical Repair of Musculoskeletal Structures

The following investigation has been conducted under this work unit:

STUDY NO. 1 Nerve repair in cats: grafts vs tension
Nerve repair in Cynomologus macaque monkeys

The ulnar nerve of the domestic cat was used as a model for repair of lacerated peripheral nerves. Sixteen cats underwent bilateral ulnar neurorrhaphy after a 2-cm segment of the nerve was removed. By using microsurgical methods, one side was repaired by an epineurial technique under tension, and the other side was repaired by using multiple sural nerve grafts. All cats were evaluated for return of function in the forelegs 6 months following nerve repairs. There was no statistical difference between these 2 techniques in overcoming segmental nerve defects in cats, which suggests that moderate tension is neither worse, nor better, than inserting avascular grafts. The results of neither technique were as good as those seen with end-to-end repair of a nerve when no segment defect existed. The rate and morphometric pattern of axon regrowth are being examined by sequential biopsies of the ulnar nerves of rhesus monkeys at weekly intervals following neurorrhaphy.

The ulnar nerve of the Cynomologus monkey was used as a model for repair of lacerated peripheral nerves. Sixteen monkeys underwent bilateral ulnar nerve transsection and resection of 0, 1, 2, or 3 cm of the ulnar nerve in the mid forearm. By using our microsurgical techniques, one side was repaired by a standard epineurial technique under tension and the contralateral side was repaired by using multiple interfascicular sural nerve grafts. Five months after the neurorrhaphies, the animals were evaluated for return of function. Evaluation included axon counts proximal and distal to the neurorrhaphies, as well as in the mid-graft segment on the grafted side and in the appropriate digital nerves in the hand. Additional evaluation included the weights of the reinnervated intrinsic muscles of the hand and histologic evaluations of the neuromas and reinnervated muscles. Clinically, all neurorrhaphies healed and produced reinnervation of the hand intrinsic muscles. Currently, the axon counts and histologic studies are being accomplished. The results indicate that more important than the type of repair is the amount of nerve tissue lost during the initial injury in determining the end result following neurorrhaphy.

BODY OF REPORT

WORK UNIT NO.	015	Animal Models for Surgical Repair of Musculoskeletal Structures
STUDY NO.	1	Nerve repair in cats: grafts vs tension Nerve repair in Cynomologus macaque monkeys

PROBLEM

Peripheral nerve injuries are common in both combat and noncombat military accidents. Many of the war injuries from the Vietnam conflict included severe damage to the peripheral nerves of the upper and lower extremities. During one 24-month period, 54% of all casualties in military hospitals had such injuries. Although our technical capabilities in the surgical repair of peripheral nerves have progressed greatly during the last several years, we still do not have a good method of managing segmental nerve defects. Tension at the repair site has been considered detrimental to nerve regeneration and healing. Consequently, the use of a multiple nerve graft has been advocated. Problems of repairing a nerve under tension (where joints must be flexed, nerves must be mobilized, and vascularity is diminished) are not completely overcome by the use of multiple nerve grafting procedures (in which an avascular unmatched segment is used to bridge the defect and relieve tension). Intrafascicular grafting not only results in the interposition of an avascular segment which loses all endoneurial elements and structure, but this technique also requires 2 separate neurorrhaphies which regenerating neurites must cross. We know of no evaluation comparing nerve repairs with tension to those neurorrhaphies which have been done with the use of multiple grafts. These studies critically compare, by objective evaluation, epineurial end-to-end repairs with tension to interfascicular grafts without tension following loss of a nerve segment.

RESULTS AND DISCUSSION OF RESULTS

We have previously described an experimental model for peripheral nerve repair by using both ulnar nerves of domestic cats comparing epineurial versus perineurial fascicular techniques. In the present study, 16 domestic cats underwent bilateral resection of a 2-cm length of the ulnar nerve proximal to the medial humeral epicondyle. One nerve was sutured under tension with size 8-0 nylon by using an epineurial technique. The other nerve was repaired by using a multiple caudal cutaneous sural graft that eliminated all tension at both suture lines. Size 10-0 nylon was used to suture the grafts. Microsurgical technique was used for all nerve repairs. Six months after the nerve sutures, cats were evaluated for comparison of return of function. Subjective evaluation included observation of gait, ability to fan claws (intrinsic

function), and withdrawal from pin prick (sensation). Objective evaluation included efficiency and maximum strength of the ulnar innervated flexor muscles, weight of the flexor carpi ulnaris muscle, and regrowth of myelinated nerve fibers by total axon counts proximal and distal to the repairs.

Evaluations have been completed and statistically analyzed. There was no statistical difference between these 2 techniques in overcoming segmental nerve defects in cats; these findings suggest that moderate tension is no worse and no better than inserting avascular grafts. When compared to an initial study where nerves were repaired primarily without tension, we found that all animals with segmental defects had less return of function than those animals which had no segmental defect but merely an acute laceration and end-to-end repair. To determine the rate and morphometric pattern of axon regrowth following nerve laceration and repair, the ulnar nerves of 6 rhesus monkeys were severed and repaired primarily. At one-week intervals beginning 7 days after the neurorrhaphies, the nerves were biopsied and prepared for light and electron microscopic examination. Analysis of these sections has demonstrated that axon sprouting begins immediately after transection and repair with the neurite sprouts passing rapidly into the distal stump. The sequential nature of the Wallerian degeneration has been well demonstrated, and the regrowth and remyelinization of the axon sprouts are clearly demonstrated by the series of electron photomicrographs.

Sixteen Cynomologus macaque monkeys underwent resections of 0, 1, 2, or 3 cm of both ulnar nerves in the mid forearm. On one side, a repair was accomplished (using 8-0 nylon) by standard epineurial technique under varying amounts of tension as determined by the amount of defect. The contralateral nerve was repaired by using multiple sural cutaneous nerve grafts that eliminated all tension at both suture lines. Size 10-0 nylon was used to resuture the grafts. A microsurgical technique using appropriate magnification was used for all nerve repairs. Five months after the nerve sutures, the monkeys were evaluated for return of function. Subjective evaluation included inspection of the neuromas and stimulation of the ulnar nerves proximal to the neurorrhaphies, and evaluation of the amount of contraction in the hand intrinsic muscles. Objective evaluation included weights of the ulnar innervated hypothenar intrinsic muscles in the hands, as well as the axon counts of myelinated nerve fibers proximal and distal to the neurorrhaphies and in the reinnervated digital nerves in the ring and little fingers.

Objective evaluations have been completed and all neurorrhaphies had healed and had produced sufficient reinnervation to allow no detectable difference in gross contraction of the ulnar innervated intrinsics.

The histologic studies on the neuromas and the hand intrinsic muscles have shown significant scarring distally into the digital nerves

regardless of the repair technique. Axon counts have indicated satisfactory reinnervation into the distal stump without statistical difference between the two repair techniques.

CONCLUSIONS

Although we do not as yet have a satisfactory answer to the management of segmental defects of peripheral nerves, we have demonstrated that nerves repaired without tension, when compared to those with segmental defects, have a greater return of function. Based on the electron microscopic study of nerve regeneration, it appears that neurite sprouting occurs immediately and without a significant delay, as has been proposed historically. From these findings we conclude that the ideal nerve repair is one performed as soon as possible after the injury, without tension, without grafts, with atraumatic technique, and with appropriate alignment of the fascicular nerve ends.

The most important factor influencing the end result is not the surgical technique, but rather the amount of segmental defect which occurred at the time of injury. From this study it appears that, depending on the amount of segmental defect, the surgeon should meet the previously established criteria of performing a neurorrhaphy as soon as possible after the injury without tension, with grafts only if necessary, with atraumatic technique, and with appropriate alignment of the fascicular nerve ends.

RECOMMENDATIONS

From these studies it is apparent that other factors, such as immunologic responses, the role of nerve growth factor, postoperative immobilization techniques, and further work on surgical technique, must be studied in order to gain more knowledge about the management of peripheral nerve injuries.

PUBLICATIONS

1. RODKEY, W.G., H.E. CABAUD, and H.R. McCARROLL. Neurorrhaphy after loss of a nerve segment: Comparison of epineurial suture under tension versus multiple nerve grafts. J Hand Surg (in press)

2. CABAUD, H.E., W.G. RODKEY, and H.R. McCARROLL. Peripheral nerve repairs. Studies in higher non-human primates. J Hand Surg 5:201-206, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE 6309	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. Change	U	U		NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	088 APC HL12
b. CONTRIBUTING	62772A	3S162772A814	00	016
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)*

(U) Studies in Combat Fracture Healing

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 012600 Pharmacology; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 08	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	0.6	21
FISCAL YEAR CURRENT 81	0.3	13

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Combat Injuries; (U) Fractures; (U) Ligamentous Injuries; (U) Trauma; (U) Surgery

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Fractures and ligamentous injuries due to combat frequently result in delayed healing and permanent disability. Prolonged hospitalization and multiple surgical procedures delay return to duty, and eventual medical separations are common sequelae to such injuries. Multiple systemic and mechanical factors are known to retard fracture and ligament healing, but considerable controversy still exists about how fracture and ligament healing can be accelerated. Biochemical alterations and various surgical modalities will be investigated. The results will be transferred into management principles and techniques for combat fracture healing.

24. (U) Twelve dogs underwent acute anterior cruciate ligament repair with augmentation utilizing the medial third of the patellar tendon. They were evaluated for function and mechanical strength at 4 and 8 months after the repairs. Additionally, augmentation utilizing a biodegradable cruciate ligament is currently in progress. Rats were exercised at endurance levels of differing frequency and duration, and then the anterior cruciate ligaments were tested for changes in tensile strength.

25. (U) 79 10 - 80 09 All repaired and augmented anterior cruciate ligaments healed and provided normal function and satisfactory strength. The ligaments tested at 8 months were stronger than those tested at 4 months. The ligaments augmented with a biodegradable splint have likewise healed, but the study is still in progress and final evaluation has yet to be completed. All exercise regimens in rats proved beneficial to the strength and stiffness of the anterior cruciate ligaments, but those exercised at high frequency (daily) and lower duration (30 minutes rather than 60 minutes) had the greatest increase in strength and stiffness.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 016 Studies in Combat Fracture Healing

The following investigations have been conducted under this work unit:

STUDY NO. 3 Evaluation of repair techniques in treating avulsion fractures and injuries of the anterior cruciate ligament

EX-4 The effect of exercise and interval training upon anterior cruciate ligament strength

Eleven dogs underwent transection of the anterior cruciate ligament at the femoral origin of one stifle (knee) joint. The anterior cruciate ligaments were repaired in a conventional manner and augmented by transferring the medial one-third of the patellar tendon and inserting it into the lateral femoral condyle. The repairs were evaluated 4 and 8 months postoperatively. All repaired and augmented anterior cruciate ligaments in this series healed satisfactorily to provide clinical and functional stability of the knee joints. Histologic evaluation showed that by 8 months the repaired and augmented anterior cruciate ligaments had healed by bony ingrowth, thus interstitial failure occurred during failure testing. The transferred patellar tendon provided additional blood supply, splinted the anterior cruciate ligament to allow healing, and increased the strength of the repaired complex. Based on the excellent results of augmentation of repaired anterior cruciate ligaments utilizing the patellar tendon, a biodegradable prosthetic splint has been developed. This biodegradable cruciate provides support to allow the repaired anterior cruciate ligament to heal. At this time, 6 dogs have undergone transection and repair of their anterior cruciate ligament with augmentation utilizing the biodegradable splint and in all cases the anterior cruciate ligaments have healed and provided functional stability for the knee.

STUDY NO. 3, EX-4. Seventy-five rats were divided into a control and 4 exercise groups of differing frequency and duration. After 8 weeks of endurance-type exercise on a motorized treadmill, the rats were sacrificed and the anterior cruciate ligaments were tested to failure. This study has shown that endurance-type exercise is beneficial to the anterior cruciate ligament as both strength and stiffness are increased, and functionally the ligament remains unchanged by the exercise.

BODY OF REPORT

WORK UNIT NO.	016	Studies in Combat Fracture Healing
STUDY NO.	3	Evaluation of repair techniques in treating avulsion fractures and injuries of the anterior cruciate ligament

PROBLEM

Incompetence of the anterior cruciate ligament and the resulting rotatory instability of the knee is a militarily devastating handicap. A significant percentage of soldiers who sustain anterior cruciate ligament injuries in training or combat, develop knee instability and require medical separation regardless of methods of treatment. Although excellent functional, anatomical, and biomechanical studies of the anterior cruciate ligament have been reported, there is still considerable disagreement as to whether a ruptured or avulsed anterior cruciate ligament should be repaired, discarded, replaced, or ignored. Based on our results in our initial study, where primary repairs were accomplished in the proximal and distal portion of the anterior cruciate ligament, this current study will evaluate the results of primary repairs of the anterior cruciate ligament augmented with the medial third of the patellar tendon. Based on the results of supplemental autogenous grafting in the current studies, we are currently evaluating the role of biodegradable synthetic materials in repairing, augmenting, or replacing the injured anterior cruciate ligament.

RESULTS AND DISCUSSION OF RESULTS

Eleven dogs underwent transection and repair of the anterior cruciate ligament at the femoral origin. The ligament was augmented by transferring the medial one-third of the patellar tendon and inserting it into the lateral femoral condyle. The repairs were evaluated at 4 and 8 months postoperatively, and all repaired anterior cruciate ligaments healed clinically providing excellent functional stability in all animals. Instron testing of the repaired and augmented anterior cruciate ligaments showed maximum strength at 4 months of 46.2 ± 10.9 kg_f and at 8 months of 64.3 ± 14.3 kg_f as compared to the control of 122.7 ± 11.6 kg_f. Histologic evaluation showed that by 8 months the repaired and augmented anterior cruciate ligaments had healed by bony ingrowth, thus interstitial failure occurred during Instron testing.

Six dogs have thus far undergone transection and repair of the anterior cruciate ligament at the femoral origin with augmentation utilizing a biodegradable splint. Evaluation has been carried out at 4 months in 5 of the dogs, and in all cases the anterior cruciate ligaments have healed and provided clinical and functional stability. Instron testing

of the repaired ligaments has shown return of strength to approximately the same range as with augmentation by the patellar tendon.

CONCLUSIONS

It is apparent that the transferred patellar tendon has provided the repaired anterior cruciate ligament with the opportunity to heal and regain functional competence. Additional blood supply was grossly evident with new blood vessels passing from the patellar tendon to the anterior cruciate ligament. The transferred patellar tendon acted as an internal splint to provide support and knee joint stability while the cruciate healed. We believe that early stress on a repaired anterior cruciate ligament may be extremely deleterious. Finally, in this study the transferred patellar tendon truly augmented the strength of the anterior cruciate ligament with one repaired complex being stronger than the opposite control anterior cruciate ligament. Likewise, the biodegradable splint has provided support and protection for the anterior cruciate ligament, allowing it to heal and return functional stability to the knee joint.

RECOMMENDATIONS

Based on our excellent results with augmented primary repairs utilizing the patellar tendon, clinical trials utilizing the technique developed at the Letterman Army Institute of Research have begun in the treatment of patients at the U.S. Army Military Academy, West Point, New York. Since the last annual report, a synthetic biodegradable cruciate ligament has been developed and studies are currently in progress to evaluate the long-term results from augmentation of repaired anterior cruciate ligaments with a biodegradable splint. Since this splint is biodegradable, it is recommended that additional postoperative evaluation be carried out both at more frequent intervals after surgical repair, e.g., 1, 2, 3, and 4 weeks, and at longer intervals, e.g., 8 and 12 months after repair. Additionally, alteration in the composition of the biodegradable splint may allow for alteration in the strength and longevity of the ligament within the knee joint.

PUBLICATIONS

1. CABAUD, H.E., J.A. FEAGIN, and W.G. RODKEY. Acute anterior cruciate ligament injury and augmented repair. Experimental studies. Am J Sports Med 8: 395-401, 1980.

2. CABAUD, H.E., W.G. RODKEY, and J.E. FITZWATER. Medial meniscus repairs: An experimental and morphological study. Am J Sports Med, in press.

Studies in Combat Fracture Healing (Cont)

3. CABAUD, H.E., W.G. RODKEY, and J.A. FEAGIN. Experimental studies of acute anterior cruciate ligament injury and repair. Orthop Trans 3: 98, 1979.

STUDY NO. 3, EX-4 — The effect of exercise and interval training upon anterior cruciate ligament strength

PROBLEM

Since the anterior cruciate ligament is particularly susceptible to injury and often requires surgical repair, it is imperative to determine whether or not exercise and interval training would strengthen the anterior cruciate ligament and therefore protect it against injury. A significant percentage of soldiers who sustain anterior cruciate ligament injuries in training or combat develop knee instability require medical separation regardless of methods of treatment.

RESULTS AND DISCUSSION OF RESULTS

Seventy-five rats were divided into a control and four groups for exercise of differing frequency and duration. After 8 weeks of endurance-type exercise on a motorized treadmill, the rats were sacrificed and the anterior cruciate ligaments were tested to failure on an Instron Materials Testing Machine at a strain rate of 95% per second. Of the 121 ligaments tested, 119 failed by pure interstitial failure. There was significant increase in both the strength and stiffness of the anterior cruciate ligaments in the exercised rats, but those rats exercised more frequently (daily versus every other day) and for shorter duration (30 minutes rather than 60 minutes) had the greatest increase in strength.

CONCLUSIONS

We can conclude from this study that 1) endurance-type exercise has a generally positive effect on the strength and stiffness of the anterior cruciate ligament; 2) the greatest increase in strength and stiffness is produced from high frequency, low duration exercise and the minimum changes from low frequency, high duration exercise; and 3) independently longer or less frequent exercise may decrease the generally positive increase in strength and stiffness that develops after daily short-duration endurance exercise.

RECOMMENDATIONS

Based on the findings in this study, we suggest that perhaps it would be appropriate to change training regimens for both athletes and soldiers in efforts to provide the most significant increases in strength

for their static supporting ligaments of the knee joint. Certainly, rehabilitation programs following surgical repair of the anterior cruciate ligaments should be modified to require high frequency, low duration exercise which appears to increase strength of the ligament most effectively. Additional studies are needed to evaluate further the effects of exercise on the strength of ligaments.

PUBLICATIONS

1. CABAUD, H.E., A. CHATTY, V. GILDENGORIN, and R. FELTMAN. The effect of exercise on the strength of the rat anterior cruciate ligament. Am J Sports Med 8: 79-86, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6317	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AD	089 JL004
b. [redacted]	62772A	3S162772A814	00	018
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)
(U) Development of Optimal Blood Products

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
002300 Biochemistry; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 01	82 10	DA	C. IN-HOUSE

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	4.4	165
FISCAL YEAR CURRENT 81	3.8	132

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NAME: POC:DA

21. GENERAL USE
Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Blood Storage; (U) Adenine; (U) Optional Additive Solutions

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Forward resuscitation of the wounded soldier requires that front line medical units maintain an adequate supply of viable, functional whole blood or packed red cells. This inventory must be available in spite of large fluctuations in usage, and delays, limitations, or interruptions in normal supply lines. This dictates that stored blood has the longest possible shelf life and can be of the highest quality. The work unit addresses the development of extended liquid storage of blood (42-100 days) as well as the improvement of the oxygen transport function of the stored blood.

24. (U) Chemicals known to improve red cell adenosine triphophate (ATP) (survival) and 2,3 diphosphoglycerate (2,3-DPG) (function) will be evaluated singly and in combination using modern optimization techniques. Maximally effective formulations of citrate phosphate dextrose (CPD) adenine and optimal additive systems will be developed. The 2,3-DPG maintenance problem will be studied and the membrane integrity limits of long term liquid storage defined.

25. (U) 7910-8009. Studies were done to confirm that CPDA-2 blood could be held 8 hr at 22 C prior to component preparation. Optimal additive system (OAS) solutions were evaluated including a saline-adenine-glucose solution, and ascorbate-2-phosphate added to CPDA-1 whole blood. Both maintained red cells for 42 days while the latter also maintained elevated P_{50} via 2,3-DPG preservation. The plasma hemoglobin levels in OAS were also examined. Long term solution stability studies of ASP and DHA were started.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 018 Development of Optimal Blood Products

An in vitro evaluation of an initial 8-hour hold with CPDA-2 anticoagulated blood indicated that a third of the 2,3-DPG was lost but that all other parameters remained unchanged from controls. Two Optional Additive Systems (OAS) were also studied. These consisted of ascorbate-2-phosphate (AsP) added to CPDA-1 whole blood and an OAS solution of saline, adenine, and glucose (3 concentrations) added to CPD-anticoagulated packed red cells. After 42 days of storage with either of these OAS solutions the red cell adenosine triphophate (ATP) levels were well maintained which suggests that these cells will have adequate viability when reinfused into humans. The AsP in CPDA-1 system also showed elevated 2,3-DPG and P_{50} values to at least 21 days of storage (P_{50}=30). The magnitude, causes, and inhibition of elevated plasma hemoglobin which occurs during extended red cell storage were evaluated. New more definitive solution stability studies were started for potential additives AsP and dihydroxyacetone (DHA). Talks were held with two drug companies concerning the need to develop OAS and saline-adenine-glucose systems for clinical testing.

BODY OF REPORT

WORK UNIT NO. 018 Development of Optimal Blood Products

PROBLEM

Military blood banking differs from its civilian counterpart because of unique logistic limitations imposed in combat situations. In a civilian setting blood is drawn, stored under "ideal" conditions, and used in a geographically contained community at a relatively predictable rate. Under these conditions, blood shortages are minimal and loss due to outdating is less than 10%. The wartime use of blood in the Army may be illustrated by the Vietnam experience, which is probably a best-case example. The blood used in Vietnam was drawn in CONUS and had CPD anticoagulant added. It had a 21-day dating period. The time required to process and ship this blood to field medical units was 7 to 14 days, which left only 7 to 14 days of shelflife remaining. Due to limited shelflife and the large fluctuation in casualty rate, outdating was possibly as high as 50%, while inventories were dangerously low in many instances. These problems could have been largely overcome if the shelflife of blood had been 35 or 42 days. In future conflicts, the U.S. may not have air superiority, thus logistic problems in all areas of supply, including fresh blood and blood products, will be compounded. To support the wounded soldier with available blood products, it will be imperative to be able to store blood for extended periods of time. In addition, it is essential that the stored blood maintains its functional qualities. These ends can be met by the development of new systems for blood storage that extend the shelflife (viability) and improve the oxygen-delivering quality of red cells. A significant step in this direction was taken with the development of CPDA-1 anticoagulant which allows for the 35-day storage of whole blood or packed cells of hematocrit not over 80. New efforts are underway to extend blood storage beyond 35 days, and also to improve the quality of long-term stored blood. At this time, two specific studies are underway: a) optimization of CPD-adenine, and b) development of an Optional Additive System (OAS). The development of CPDA-1, while offering a significant improvement in blood storage, does not produce the optimum results in red cell storage that is attainable with a glucose-adenine mixture. Two new formulations of CPD-adenine (CPDA-2 and CPDA-3), which are close to optimal for packed cell storage, were evaluated (in vitro). These studies were summarized in the Annual Research Progress Report, 1979 (page 158).

CPDA-2 is a significantly superior product, based on in vitro tests, when compared to CPDA-1. Red cells were stored in CPDA-2 for periods up to 56 days. The best approach to extended quality storage of red blood cells is by use of a specific solution for addition to packed red cells. This approach is termed an "Optional Additive System." Solutions are being developed and tested (in vitro) which allow for extended storage of packed red cells, and at the same time improve the functional quality of these cells by maintaining the concentration of red cell 2,3-DPG. The development

of these systems will provide military blood banking with the capability to (a) store red blood cells for extended periods of time beyond 35 days, (b) improve the functional qualities of these cells, i.e. their oxygen off-loading characteristics, by maintaining normal P_{50}, and (c) make available for separate use, fresh plasma components in maximum quantities, free of adenine or other additives.

RESULTS AND DISCUSSION OF RESULTS

Studies with CPDA-2 were pursued in two areas. The effects of a room temperature 8-hour hold between phlebotomy and component preparation were examined. This 8-hour hold causes a mean loss of 30% of the red cell 2,3-DPG and a mean increase of 10% in cell ATP. These differences are reflected throughout 42 days of red cell storage. Other red cell parameters such as pH, plasma hemoglobin, and glucose utilization are not significantly affected. Clinical trials are being done with the CPDA-2 anticoagulant (see WU 004), DAOE6087. In vitro studies done as part of these trials have been expanded to include methylene blue uptake, fluoroscein diacetate conversion, and samples have been saved to measure adenylate energy charge. These assays will be correlated with red cell survival in an attempt to find additional in vitro correlates to red cell viability.

Studies have continued with the development of OAS solution using dihydroxyacetone (DHA) and/or ascorbate-2-phosphate (AsP) to maintain red cell O_2 delivery function. The study of long-term solution stability of AsP was aborted during the previous fiscal year due to loss of technical support. The analytical procedures also needed to be refined. Using improved techniques, we restarted these studies. A rapid, accurate, high pressure liquid chromatography (HPLC) assay for AsP and its breakdown products was developed in our laboratory. AsP solutions in saline and saline-adenine-glucose (SAG) were stored as individual samples in 50 ml transfer packs, each sealed and heat processed (to prevent mold) in ½ pt canning jars. These solutions will be assayed over a 3-year period. After 3 months, no loss of stability is seen in the AsP at room temperature. Similar studies are being planned to reevaluate the solution stability of DHA, but the HPLC separation has not yet been perfected. A new simple microassay for plasma hemoglobin has been developed which gives a stable linear response in the range of 5-2000 mg/dl. It is based on the cynamet-hemoglobin procedure and is being used to evaluate plasma hemoglobin in OAS stored samples. OAS stored red cell can generate several hundred mg/dl plasma hemoglobin. By storing the red cells with 1% epsilonamino caproic acid (EACA) red cell hemolysis can be reduced up to 80%. These data confirm a similar observation that EACA inhibited white cell proteases which caused cell lysis. However, in another study, we used a split bag OAS experiment in which half the cells were filtered (Teruno filter) to remove 99% of the white cells within 1 hour of collection. Filtering did not retard hemolysis, indicating that either the white cell proteases were

released before or during filtration, or that the EACA-white cell protease theory is in error. EACA-treated red cells improved ATP concentrations compared to the controls without EACA. Studies were also done to investigate an OAS containing saline, 0.25 mM adenine and glucose (1X, 1.25X, 1.5X of CPD), frequently called the SAG system. All glucose concentrations studied produced similar results with red cell ATP levels remaining above 50% of T_o for 42 days of storage. One set of studies has also been done using AsP (above) as an OAS which was added to CPDA-1 whole blood to produce extended storage with elevated 2,3-DPG. Data analysis has not yet been completed on these studies, however, day-21 P_{50} values were in the low 30s, which indicated some benefit to the system. Talks were held with representatives of the blood bag industry (Cutter and Fenwal) concerning possible joint ventures to get SAG and/or OAS solutions developed and into clinical testing in the near future.

CONCLUSIONS

CPDA-2 blood can be held for 8 hours before component preparation without apparent harm to red cell viability.

Further testing of OAS solutions confirms their potential as the next logical step forward in providing improved blood products. These red cell products should possess both extended storage capability and improved oxygen-delivery function. Plasma hemoglobin rises in extended red cell storage, for reasons which are not yet defined. When analyzed in terms of hematocrit and cell lysis, the rise in plasma hemoglobin represents less than 1% cell lysis and should be considered more of an aesthetic than a clinical problem.

RECOMMENDATIONS

OAS solutions, perhaps with SAG as a first step, should be developed further in close cooperation with the drug companies, so that clinical testing can be started within the next year or two. The development of technologies to provide long-term stored red cells with improved oxygen delivery is a realistic mid-term goal (2-3 years) that should be pursued in conjunction with the civilian community to insure its availability for military needs.

PUBLICATIONS

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2. PECK, C.C., G.L. MOORE, and R.B. BOLIN. Adenine in blood preservation. CRC Reviews in Clinical Laboratory Science, 1981. (in press)

3. PECK, C.C., G.L. MOORE, V. LICKO, R.B. BARRET, and L. LENERT. The design of optimal blood preservation systems. Clin Res 28:36-37, 1980

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6. MOORE, G.L., M.E. LEDFORD, M.R. BRUMMELL, and K.A. UNRUH. Red cell storage for 56 days in modified CPD-adenine: an in vitro evaluation. Transfusion, 1980 (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DA OE 6090	80 10 01	DD-DR&E(AR)636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AC	090 JL05
b. [redacted]	62772A	3S162772A814	00	019
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Procode with Security Classification Code)*

(U) Investigation of Cell-Free Resuscitating Solutions

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

008800 Life Support; 003500 Clinical Medicine; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 03	CONT	DA	C. IN-HOUSE

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	5.9	261
	CURRENT 81	15.6	580

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procode EACH with Security Classification Code) (U) Acute Resuscitation; (U) Stroma-Free Hemoglobin; (U) Blood Substitute Solutions; (U) Hemorrhagic Shock

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procode text of each with Security Classification Code.)

23. (U) The objective of these studies is to develop and evaluate an effective hemoglobin solution as blood substitute that can be stockpiled and used in forwared resuscitation for military combat casualties. Hemoglobin, free of cell constituents, can provide the basis for an ideal resuscitating fluid for the severely wounded soldier. It has advantages over plasma expanders since oxygen transport, long term stability, and reduced volume and mass compared to blood are possible.

24. (U) Formulations of solutions of hemoglobin which do not cause adverse effects when administered in vivo in animal models are being investigated. In order to overcome the limitations of short intravascular retention and high oxygen affinity, inter and intramolecular hemoglobin modifications are being studied: a) phosphorylated sugars, b) gluteraldehyde, c) phosphoenolpyruvate and d) phosphorylated aldehydes. In addition to efficacy studies, safety is being evaluated in vitro and in vivo.

25. (U) 7910-8009. In the studies on the modification of hemoglobin a pyridoxalated-polymerized product has been obtained with higher P_{50} (20-22 torr) and longer vascular retention time in in vivo ($T_{\frac{1}{2}} \sim 25$ hr) than unmodified hemoglobin. The modified hemoglobin does not demonstrate in vitro coagulant activity and, when used as a blood substitute in a total blood replacement in rats, can maintain vital signs. All animal survival morphological analysis of tissue show normal cell structure. Intramolecular modification of the hemoglobin molecule are also being investigated. All but the phosphorylated dialdehydes have been eliminated as being too slow or too nonspecific reacting with hemoglobin. The dialdehydes result in rapid, high yield reactions cross-linking dimers to tetramers and also improving oxygen transport. Coagulation studies reveal serious coagulant activity in unmodified hemoglobin preparations due to soluble lipids.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3S162772A814	Military Trauma and Resuscitation
WORK UNIT NO.	019	Investigation of Cell-Free Resuscitating Solutions

The following investigations have been conducted under this work unit:

STUDY NOS.	1,2,4,5	Preparation of hemoglobin, in vivo evaluation, pharmacokinetics, and effects of hemoglobin on organs
STUDY NO.	6	Molecular modifications of hemoglobin

STUDY NOS. 1,2,4,5 and 6. Attempts to modify the hemoglobin molecule have been continued with the objective of obtaining a product with lower oxygen affinity and longer vascular retention time than unmodified hemoglobin. A pyridoxalated-polymerized hemoglobin has been prepared with higher P_{50} (20-30 torr) and a longer intravascular life in vivo ($T_{\frac{1}{2}} \sim 25$ hours) than unmodified hemoglobin. The optimal conditions for the preparation of modified hemoglobin have been studied. The modified hemoglobin does not demonstrate coagulation activity by four conventional in vitro coagulation tests but the toxicology of hemoglobin related to the development of in vivo coagulopathies has not been resolved. In vivo evaluation of pyridoxalated-polymerized hemoglobin has shown that a solution of this compound can be used as a blood substitute in a total blood replacement in rats and can maintain vital signs. All animals survived. Morphological analysis of tissues such as liver and kidney show normal cell structure and no sign of hypoxia. Modification of hemoglobin by a variety of reagents under many conditions was attempted. Phosphorylated sugars reacted too slowly, and gluteraldehyde lacked specificity. Of all the modifications to date, the use of the phosphorylated dialdehydes appears to hold the most promise. Formed from readily available phosphorylated ribose derivatives (e.g. adenine nucleotides) reacted with sodium periodate, these compounds specifically crosslink hemoglobin with significantly less alteration in hemoglobin structure than occurs with the use of gluteraldehyde. The phosphorylated dialdehydes address, in a single step reaction, both the limitations of a high oxygen affinity and a rapid renal clearance while minimizing changes to the otherwise desirable features of unmodified hemoglobin.

BODY OF REPORT

WORK UNIT NO.	019	Investigation of Cell-Free Resuscitating Solutions
STUDY NOS.	1,2,4,5	Preparation of hemoglobin, in vivo evaluation, pharmacokinetics, and effects of hemoglobin on organs

PROBLEM

For several decades many investigators have been involved in the development and evaluation of resuscitating solutions as blood substitutes. There are several reasons for pursuing these studies. As a resuscitating fluid blood appears to be ideal for transfusions and it is widely used in fluid replacement therapy for hemorrhagic shock. However, blood has a limited storage life, which at present does not exceed 35 days. It requires specialized expertise and equipment for its collection, transportation, storage, and pre-transfusion preparation such as typing and cross-matching. Furthermore, problems occur when using blood for transfusions. Therefore, finding a suitable resuscitating solution which would alleviate or minimize such problems would be advantageous particularly to personnel in combat.

In military conflicts, the conventional approach to the care of combat casualties has been prompt evacuation of the wounded soldiers to treatment facilities and rapid access to medical assistance. This evacuation was possible in Korea and Vietnam, either because hospitals were close to a stable front line or because air superiority made helicopter evacuation possible. However, in a future war, battlefield conditions may severely hinder evacuation and care of casualties will be delayed. Therefore, the development of an effective resuscitating solution that can be administered in the battlefield acquires substantial importance.

In military field operations, requirements for transfusion frequently demand massive fluid support in areas remote from supply sources. The inability to predict when modest transfusion requirements may suddenly increase complicates fluid therapy logistics. The ability to stockpile a stable resuscitating solution capable of carrying and exchanging oxygen would minimize many of these difficulties.

It is evident that significant advantages can be gained by the development of a resuscitating solution capable of transporting oxygen, maintaining oncotic pressure, and being readily available when massive clinical transfusions are required. Stringent requirements must be met by a resuscitating solution in order to be effective. As a blood substitute, this solution not only must be capable of restoring vital functions, but also must not elicit permanent adverse effects when administered to victims in mass casualties. Furthermore, it must be uniquely suited to fulfill the supply, storage, and transportation requirements for field use in combat situations.

Investigation of Cell-Free Resuscitating Solutions

A solution of hemoglobin has the potential to fulfill the characteristics required for a blood substitute. Many investigators have stressed several advantages of this solution as compared with other resuscitating fluids or plasma expanders. Hemoglobin is a component of normal blood, can be prepared from outdated human erythrocytes, does not require typing or cross-matching before it is used, is capable of transporting oxygen, has oncotic activity, has lower viscosity than blood, does not cause micro-aggregates, and may not induce immunologic reaction. Furthermore, hemoglobin is highly soluble in physiological solutions and can be stored for extended periods of time.

The potential value of hemoglobin solutions as an oxygen-carrying blood substitute has also been recognized in some special situations. (1) This solution could be used in the treatment of hemorrhagic shock in circumstances where compatible blood is not available or where constriction of the capillary vessels in the microcirculation would dictate the use of a fluid with a lower viscosity than blood for normovolemic hemodilution. (2) It could be helpful in the military field operating room when prolonged and continued blood loss occurs. Until bleeding is under control, hemoglobin solutions could be used, thus saving a large volume of donor blood which could be more efficiently utilized later. (3) In open heart surgery, hemoglobin solutions could be of great advantage in priming the pump and/or maintaining circulation during surgery, again saving the patient's blood without any mechanical stress, for better utilization at the end of surgery. (4) Hemoglobin solution can be used as a perfusate to preserve various organs for long periods of time in a normothermic environment, and can maintain the normal oxygen tension and oncotic pressure necessary during preservation. (5) In metabolic studies, solutions of hemoglobin can be formulated with the required components and used in organ perfusion allowing results which are unaffected by background compounds which are present when blood is used. (6) In veterinary medicine, hemoglobin solutions could become important for animal transfusions since animal blood banks do not exist.

The problem of developing an effective blood substitute is pertinent not only to military combat casualties, but also to civilian casualties.

RESULTS AND DISCUSSION OF RESULTS

Investigations on the development and evaluation of hemoglobin solution as a resuscitating fluid have been continued. Two important limitations of the present products, namely the higher oxygen affinity and the shorter intravascular retention time of free hemoglobin as compared to hemoglobin present in the red cell, have been of great concern in our laboratory. To overcome these limitations, modifications of the hemoglobin molecule were studied. In an initial attempt, hemoglobin, prepared by crystallization as described in the Annual Research Progress Report 1975 (pages 152-156), was polymerized in the presence of glutaraldehyde. The products

obtained, although useful for their prolonged intravascular retention, demonstrated a high oxygen affinity with a P_{50} of 4-8 mm Hg, lower than 14-16 mm Hg for unmodified hemoglobin and much lower than 26-27 mm Hg for hemoglobin inside the red cell. With such higher oxygen affinity, at normal tissue P_{O_2}, practically no oxygen would be released to the tissues and the hemoglobin solution would be reduced to the role of a plasma expander. In other studies, the hemoglobin molecule was first coupled with a low molecular weight phosphate compound, pyridoxal-5-phosphate (PLP), and then polymerized. The pyridoxalated-polymerized hemoglobin thus obtained demonstrated a higher P_{50} (20-23 torr) and a longer vascular retention time in vivo ($T_{\frac{1}{2}} \sim 25$ hours) than unmodified hemoglobin. The optional conditions for the preparation of this modified hemoglobin have been investigated. In the pyridoxalation reaction a molar ratio of 4:1 PLP/hemoglobin and subsequent treatment with sodium borohydrite ($NaBH_4$) with a molar ratio of 20:1 $NaBH_4$/Hb yielded a phosphate-hemoglobin complex with the highest P_{50}. In the polymerization process, a molar ratio of 5:1 glutaraldehyde/hemoglobin gave the best results. Preliminary results show that the use of $NaBH_4$, a mild reducing agent, in promoting a covalent bond between PLP and hemoglobin does not affect the structure or the function of the hemoglobin molecule, as determined by methemoglobin content, P_{50}, n-value (Hill's coefficient), and oxygen-carrying capacity. In the pyridoxalation and polymerization reactions, excess quantitites of PLP, $NaBH_4$, and gluteraldehyde were removed by dialysis procedures.

Only the Kaoline coagulation test (KCT) shows coagulant activity with unmodified hemoglobin but not with pyridoxalated-polymerized hemoglobin. Studies in dogs show three consistent coagulant changes after injections of unmodified hemoglobin: early anticoagulant effect (probably hemodilution) that resolves within 48 hr, a late anticoagulant effect seen after 72 hr and lasting 7 days, and an early, transient thrombocytopenia. Two dogs receiving homologous hemoglobin developed disseminated intravascular coagulopathy (DIC).

The pyridoxalated-polymerized hemoglobin solution, prepared as described above, was evaluated in vivo by exchange transfusing rats to total blood replacements, i.e. hematocrit of 2-3 %. All animals thus exchanged survived, maintained normal activity and restored hematologic and physiologic parameters (hematocrit, total hemoglobin, P_{50}, oxygen-carrying capacity) to normal levels within 5 to 7 days after transfusion. As reported in the Annual Research Progress Report, 1976 (pages 131-138) animals similarly transfused with unmodified hemoglobin died approximately 5 hr after transfusion, due to the rapid disappearance of plasma hemoglobin. In the modified hemoglobin-treated rats higher levels of plasma hemoglobin and higher P_{50} were observed that in animals treated with unmodified hemoglobin. In different groups of rats, morphological analyses of tissues, such as liver and kidney, were done at 12 and 24 hr after 75% blood replacement with a solution of pyridoxalated-polymerized hemoglobin.

Preliminary data by phase contrast and electron microscopy show that liver and kidney cells maintain normal structure and no signs of hypoxia are evident. In vivo evaluation of this promising modified hemoglobin is being continued to assess several clinical aspects including pharmacokinetics and tissue distribution of the infused molecules.

CONCLUSIONS

Crystalline hemoglobin solution, developed in our laboratory, has been evaluated further. Hemoglobin, because of its ability to transport oxygen and maintain oncotic pressure, could provide the basis for a useful resuscitating solution for battlefield casualties. The two important limitations of the present product, namely the high oxygen affinity (low P_{50}) and short vascular retention time of the hemoglobin as compared to the hemoglobin in the red cells, have been addressed. Modification of the hemoglobin molecule by pyridoxalation and then polymerization reactions has yielded a product with higher P_{50} and longer intravascular retention time than unmodified hemoglobin. The pyridoxalated-polymerized hemoglobin appears promising. It does not demonstrate pro- or anti-coagulant activity and, when tested in vivo in rats transfused to total blood replacement, it can support vital signs by providing transport of oxygen to tissue without any apparent adverse effects on tissue structure.

RECOMMENDATIONS

Research studies involving the modification of hemoglobin aimed at maintaining the tetrameric molecule should be intensified: such efforts can provide a stable hemoglobin compound having a longer intravascular life as well as a lower oxygen affinity. Studies on coagulation properties of modified hemoglobin should be made in vivo, and should include studies on the mechanism of hemoglobin-induced coagulopathies. Due to increasing requests for large volumes of hemoglobin solutions by several interested investigators, both military and civilian, it is strongly recommended that preparations be made to encourage a pharmaceutical company to produce hemoglobin solution in large quantities according to established specifications. The hemoglobin solution would then be available to interested investigators, including those who are supported by Army contracts.

PUBLICATIONS

1. DEVENUTO, F., H.I. FRIEDMAN, and P.W. MELLICK. Massive exchange transfusions with crystalline hemoglobin solution and subsequent replacement of hemoglobin and blood volume. Surg Gynecol Obstet 151:361-365, 1980

2. MOORES, W.Y., F. DEVENUTO, W.H. HEYDORN, R.B. WEISKOPF, M. BAYSINGER, and J.P. HANNON. Improved porcine myocardial performance during severe anemia using a stroma-free hemoglobin solution. (Abst.) Fed Proc 39:2331, 1980

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7. DEVENUTO F. Acellular oxygen delivering resuscitating fluids: hemoglobin solutions. In: Proceedings Current Concepts Combat Casualties, Washington, D.C. (in press)

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STUDY NO. 6 Molecular modifications of hemoglobin

PROBLEM

Two intrinsic characteristics of unmodified hemoglobin solution, namely, its increased oxygen affinity and rapid plasma clearance, impose distinct limitations on combat field use in the massively transfused soldier by requiring repeated infusions of a solution which has decreased oxygen delivery properties. The goals of molecular modification of hemoglobin have been to resolve these two problems and thus improve the solution for resuscitative purposes. Specific endpoints desired of modified hemoglobin remain defined as a P_{50} between 25 and 40 torr (unmodified hemoglobin P_{50} = 13-17 torr) and a plasma half disappearance time of 12 to 24 hours (unmodified hemoglobin plasma half disappearance time = 2-4 hours). Finally, these endpoints must be accomplished with a minimum of structural change to hemoglobin to minimize alterations or induction of immunologic sequellae.

RESULTS AND DISCUSSION OF RESULTS

In order to achieve a crosslinking of hemoglobin that insures both the stated limitations as well as structural integrity, a reagent needs to be specific in its reaction with hemoglobin. Because the 2,3-diphosphoglycerate pocket (2,3-DPG pocket) is a distinct region on the hemoglobin tetramer that selectively binds certain phosphorylated reagents, an ideal crosslinking reagent would have charged groups such as phosphates for binding to this region and would also have at least two reactive groups capable of forming a covalent crosslink which would bind the entire tetramer together. Such reactions in this region would bind the entire renal retention by preventing dimer formation. Further, enhanced oxygen unloading would result because the pocket would be covalently constrained in the deoxy conformation.

Three classes of molecular modifications were studied. Phosphorylated sugars, gluteraldehyde, and a new class of phosphorylated dialdehydes were all evaluated under a variety of conditions. Of these, the phosphorylated dialdehydes appear quite promising as a specific intramolecular crosslinking reagent.

Glycosylation with phosphorylated sugars was studied extensively and was shown to lack specificity in reacting with hemoglobin. Although much was learned about what reaction conditions hemoglobin will sustain, this class of compounds does not appear useful for formation of a modified hemoglobin preparation.

Gluteraldehyde crosslinking was investigated under a number of conditions with and without the presence of inositol hexaphosphate (IHP). By utilizing buffered solutions, deoxygenated hemoglobin, and IHP (as a blocking agent for the 2,3-DPG pocket) the P_{50} of gluteraldehyde crosslinked hemoglobin was improved from 4-8 torr up to 11-16 torr. However, the degree of crosslinking and denaturation as shown by isolectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was pronounced. Under no circumstances attempted could crosslinking be controlled; invariably many intermolecular aggregates formed with molecular weights exceeding 200,000. This level of crosslinking may ultimately have pronounced effects in a shock victim and large aggregates may interfere with the microcirculation and the reticuloendothelial system. Further, such extensive crosslinking almost certainly alters the surface of the modified hemoglobin which may result in undesired antigenic stimulation in the host.

Because extensive crosslinking may be undesired, a new group of reactions was studied to promote a controlled intramolecular crosslinking resulting in tetramer only. Phosphorylated ribose derivatives such as adenosine triphosphate (ATP) and phosphoribosyl-1-pyrophosphate (PRPP) were reacted with sodium periodate to form a new class of phosphorylated

dialdehydes. These were reacted with hemoglobin. In every case only a crosslinked tetrameric species was formed. Furthermore, the yields were proportional to the degree of phosphorylation. Under carefully controlled conditions, P_{50}s as high as 35 torr were achieved; however, care had to be taken to avoid oxidation of hemoglobin with residual sodium periodate. These compounds appear promising as a new class of crosslinking agents that preserve the fundamental structure of hemoglobin and improve its functional properties.

CONCLUSIONS

Of the three classes of molecular modifications studied, the controlled intramolecular crosslinking to stabilize tetrameric hemoglobin appears the best approach since the basic structure is maintained and large yields of modified hemoglobin can be obtained. In addition, the intravascular retention of hemoglobin in solution would be improved because renal excretion of monomeric and dimeric hemoglobin would be minimized.

RECOMMENDATIONS

Studies should continue with intramolecular crosslinking agents. The research efforts should be focused on defining newer agents that can stabilize tetrameric hemoglobin and, at the same time, protect the molecular function (oxygen transport properties) of hemoglobin in solution.

PUBLICATIONS

1. SCANNON, P. Molecular modifications of hemoglobin. In: Proceedings Current Concepts of Combat Casualty Resuscitation, Washington, D.C. (in press)

2. SCANNON, P. Phosphorylated dialdehydes: a new class of compounds for crosslinking of hemoglobin. (Abst.) Western Meeting of American Federation of Clinical Research, Carmel, California, February 1981

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOE 6302	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. Change	U	U		NL	YES / NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	247 APC HL13
b. [redacted]	62772A	3S162772A814	00	020
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)

(U) Response of Muscle to Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	1.4	57
FISCAL YEAR CURRENT 81	2.6	78

19. RESPONSIBLE DOD ORGANIZATION

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NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Skeletal Muscle; (U) Myoglobin; (U) Metmyoglobin Reductase; (U) Heatstroke; (U) Muscle Injury; (U) Oxygen Utilization by Muscle

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The acutely injured soldier develops negative nitrogen balance and loses muscle mass through mechanisms which are unknown. One of the factors which may be involved is myoglobin, a heme-protein which transports oxygen within muscle cells. Myoglobin and its overall metabolic relationships within the muscle cell serve as useful markers in the study of muscle injury. Injured muscle loses myoglobin into the peripheral circulation where it may cause secondary renal damage for unknown reasons. Failure of myoglobin to maintain sufficient intracellular oxygen supply may lead to decreased energy production, weakness, and failure of mechanisms upon which recovery from injury depend.
24. (U) Selected aspects of the effects of injury on muscle will be evaluated. Strategies designed to minimize and/or reverse the detrimental effects of injury on muscle will be determined. The effects of muscle injury on other body systems, including the kidney, will be studied. The relationship between myoglobin (and its associated reactions in the muscle cell) and immobilization-induced muscle atrophy, exercise-induced muscle hypertrophy, and recovery from injury will be studied. The effects of various heme proteins on the kidney will be evaluated.
25. (U) 79 10 - 80 09 The influence of dietary iron deficiency on metmyoglobin reductase, myoglobin, and other iron-containing proteins was evaluated in rapidly growing weanling rats. Iron deficiency decreased circulating hemoglobin levels, cytochrome c levels in both heart and skeletal muscle, and myoglobin levels only in skeletal muscle. Skeletal muscle mitochondrial respiration was also significantly decreased in the iron deficient animals. Iron deficiency increased the activity of methemoglobin reductase in red blood cells, but was without effect on metmyoglobin reductase in muscle. The studies demonstrate the preferential utilization of iron between and within tissues, and the varying adaptive responses to iron deficiency.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 020 Response of Muscle to Injury

The following investigations have been conducted under this work unit:

STUDY NO. 1 Studies concerning the mechanism which controls the redox state of myoglobin

STUDY NO. 2 Effect of wounding on muscle metabolism

STUDY NO. 1. Studies initiated during FY 79 in collaboration with MAJ E. Wayne Askew, MS, were continued and nearly completed. In one of these studies, the effect of dietary iron deficiency on selected aspects of heme protein metabolism in the rat was extensively evaluated. Male weanling rats were fed a control diet (45 ppm iron) or an iron-deficient diet (11 ppm iron) for 7 weeks. At the end of 7 weeks, the hemoglobin in the blood of the iron-deficient rats was 35% less, and skeletal muscle myoglobin was 20-37% less than in the control animals. The concentration of myoglobin in the heart was not appreciably diminished by iron deficiency. Cytochrome c concentration was 20% less in the heart and 35% less in the mixed-fiber gastrocnemius in the iron-deficient animals. Iron deficiency had no effect on metmyoglobin reductase activity in muscle. Iron deficiency resulted in a 60% increase in erythrocyte methemoglobin reductase activity which eventuated in virtually unmeasurable levels of methemoglobin. In the iron-deficient rats, skeletal muscle mitochondrial respiration with either pyruvate-malate or palmitylcarnitine as substrate was 17-20% less than in the control animals. This study demonstrates that dietary iron deficiency of sufficient severity to reduce blood hemoglobin and skeletal muscle myoglobin or cytochrome c also results in an impaired skeletal muscle oxidative capacity. The study also demonstrates the preferential utilization of iron, not only between tissues, but within tissues, and the varying adaptive responses to iron deficiency.

STUDY NO. 2. Branched chain amino acids (BCAA) have been implicated as an important source of calories during wound-induced hypermetabolism. In addition, several investigators have reported specific anticatabolic properties of BCAA, leucine in particular. These studies were conducted to determine the effect of muscular injury on in vitro branched chain amino acid transaminase (BCAAT) activity and the in vivo rates of BCAA oxidation. Rats were wounded by intramuscular injection of 0.5% λ-carrageenan into the hindlimbs. Six days after injury BCAAT activity (μm leucine degraded/hr/mg protein) in tissue homogenates from wounded hindlimbs was one-half of control values. In vivo BCAA oxidation, estimated by trapping $^{14}CO_2$ in the expired air from intact and wounded animals which had been injected with a trace dose of ^{14}C-U-leucine, was

the same; approximately 12% of the injected dose was expired within 6 hours in both groups. These results indicate that injured muscle actually has a reduced capacity to degrade BCAA, and that whole body utilization is not different.

BODY OF REPORT

WORK UNIT NO.	020	Response of Muscle to Injury
STUDY NO.	1	Studies concerning the mechanism which controls the redox state of myoglobin

PROBLEM

Muscle function is impaired in soldiers either directly by injury or indirectly by immobilization. In order to facilitate healing and to reverse atrophy of muscle, it is necessary to understand the mechanisms involved in exercise-induced hypertrophy and immobilization-induced atrophy of muscle. Muscle is the only tissue which contains myoglobin, the presence of which subserves functions the precise nature of which remains uncertain. Since myoglobin is a heme protein, it is presumed that its function, in part, is related to oxygen transport/storage in the muscle cell. It is postulated that myoglobin may be centrally involved in the energy dependent processes of muscle via this function as an intracellular carrier of oxygen.

Myoglobin, like hemoglobin, undergoes freely reversible oxygenation in order to carry out its oxygen transport function. Myoglobin is nearly 20 times more easily oxidized than hemoglobin. The oxidized forms of hemoglobin and myoglobin (methemoglobin and metmyoglobin, respectively) are incapable of carrying oxygen. The red blood cell possesses several enzymatic mechanisms which maintain hemoglobin in the functional reduced state. We have isolated, purified, and characterized an enzyme (NADH-metmyoglobin reductase) which actively reduces metmyoglobin in vitro. The effect of dietary iron deficiency on selected aspects of heme protein metabolism, including myoglobin and metmyoglobin reductase, was extensively evaluated in the rat.

RESULTS AND DISCUSSION OF RESULTS

Four-week-old male rats were divided into two groups of 10 rats each and fed either an iron-deficient or an iron-adequate diet for 7 weeks. At the end of 7 weeks the rats were killed by decapitation following a 12-hour fast. One blood sample was collected for serum iron determination. Another sample of blood was collected for the following: hemoglobin, hematocrit, oxygen-carrying capacity, hemoglobin oxygen affinity, methemoglobin reductase, and methemoglobin. The left quadriceps muscle group was removed and utilized immediately to prepare skeletal muscle mitochondria. The right quadriceps was removed and sectioned transversely. One-half was used immediately to prepare the crude homogenate for cytochrome oxidase assays. The remaining one-half was divided: the deep red fiber portion and the superficial white fiber portion were separated and frozen for subsequent myoglobin and metmyoglobin reductase analyses.

One-half of the left gastrocnemius (mixed fiber muscle), both solei (predominantly red fiber), and the heart were also removed and frozen for myoglobin and metmyoglobin reductase analyses. One-half of the left gastrocnemius muscle and a portion of the heart were removed and frozen for cytochrome c determinations. The right gastrocnemius and liver were removed and frozen for total iron analysis. The spleen was removed, weighed, and preserved in 10% formalin subsequent to histological examination for stainable iron by using Perl's stain.

After 2 weeks on the iron-deficient diet, hemoglobin and hematocrit values of the experimental group of rats dropped to 76-70% of the control values and remained at this level for the duration of the experiment. No significant differences existed in food intake or body weight after 7 weeks on the iron-deficient diets. Iron-deficient rats tended to have slightly smaller muscle and organ weights; these differences were significant only in the case of the quadriceps muscle and liver. There was an indication of hypertrophy of the spleen in the rats consuming the iron-deficient diet; this effect was statistically significant when the spleen weight was expressed relative to body weight.

Rats consuming the iron-deficient diet had significantly lowered blood hemoglobin (14.7 vs 9.6 g/dl), hematocrit (43.9 vs 30.4%), oxygen carrying capacity (18.1 vs 12.0 vol %), and serum iron (187.4 vs 52.0 µg/dl). Hemoglobin oxygen affinity was not influenced under the conditions of this study. Methemoglobin reductase activity was 1.8 ± 0.1 units in the control animals and 2.9 ± 0.1 units in the animals consuming the iron-deficient diet. The increased methemoglobin reductase activity in the iron-deficient animals resulted in virtually unmeasurable methemoglobin values (0.06%) which contrasts sharply with the values in control animals (0.28%).

The concentration of myoglobin in the heart was not decreased by iron deficiency; neither was the myoglobin content of the predominantly white portion of the quadriceps muscle. Myoglobin concentration was significantly decreased in predominantly red fiber or mixed fiber muscles from rats consuming the iron-deficient diet.

The concentration of cytochrome c, unlike myoglobin, was significantly decreased (20%) in heart muscle of iron-deficient rats. Cytochrome c concentration was significantly decreased (35%) in the gastrocnemius muscle of iron-deficient rats.

Dietary iron deficiency had no effect on the activity of muscle metmyoglobin reductase.

Mitochondria from the iron-deficient rats appeared to be functionally normal except for a 17-20% reduced capacity to oxidize pyruvate-malate and palmitylcarnitine substrates. The mitochondrial yield, ADP/O ratio,

and respiratory control index were not significantly influenced under the conditions of this study. Although the cytochrome c content of the gastrocnemius muscle was reduced in the iron-deficient rats, the activity of cytochrome oxidase was not diminished.

Stainable iron in the spleen was confined to the red pulp where it was localized within reticuloendothelial cells. Animals receiving the control diet had abundant stainable iron in contrast to the animals receiving the deficient diet. In these animals the small amount of stainable iron was clearly distinguished by several independent observers from the substantially larger amounts in the control animals.

CONCLUSIONS

The imposition of moderate dietary iron deficiency in young rapidly growing animals leads to a variety of biochemical changes. These changes appear despite comparable rates of food intake and growth, and similar levels of tissue protein. Iron deficiency resulted in predictable changes in a number of hematologic measurements, e.g., decreased hemoglobin, hematocrit, oxygen-carrying capacity, and serum iron. Oxygen affinity was not changed under the conditions of this study. There was a striking increase in the activity of methemoglobin reductase activity in the red blood cells of the iron-deficient animals, and a corresponding decrease (to virtually unmeasurable levels) of red blood cells methemoglobin levels. It can be argued that these are adaptive changes in response to the effects of iron deficiency, which are aimed at maximizing the function of the reduced levels of hemoglobin which are available.

Skeletal muscle myoglobin concentration was decreased in predominantly red fiber or mixed fiber muscles from rats consuming the iron-deficient diet; the concentration of myoglobin in the heart and in white fiber muscle was not changed. These results confirm those of others in which the responsiveness of myoglobin levels to dietary change is dependent on the age of the animal being studied.

Cytochrome c concentration was decreased in both the heart and skeletal muscle in iron-deficient animals. Our results confirm the findings of others with regard to the responsiveness of cytochrome c levels to dietary iron deficiency in rapidly growing animals. Our results also demonstrate a hierarchy of iron utilization within tissues since, in the heart, cytochrome c levels were reduced whereas myoglobin levels were not.

The lack of response of metmyoglobin reductase is not unexpected, since the enzyme is known not to require iron for activity. The failure of iron deficiency to influence the activity of metmyoglobin reductase contrasts sharply to the marked increase in methemoglobin reductase

activity. Despite many similarities between these two enzymes, their individuality is emphasized by different responses.

The influence of iron deficiency on mitochondrial function has not been characterized extensively. Mitochondrial respiration with pyruvate-malate or palmitylcarnitine as substrate was decreased 17-20% in the iron-deficient group. The degree of decrement in the oxidation of these two substrates was similar, which indicates that the biochemical lesion induced by iron deficiency may be common to both pathways of carbohydrate or fatty acid oxidation. The results of this study suggest a generalized impairment of mitochondrial oxidative capacity may exist in iron-deficient animals and may be responsible for the reduced work capacity which is associated.

The study demonstrates that rapidly growing young animals which are fed an iron-deficient diet will utilize the available iron in an hierarchical manner. Certain iron-containing heme proteins such as cytochrome c are decreased in a general fashion, whereas others, such as myoglobin, are decreased in certain muscles but not in others. The increase in met-hemoglobin reductase levels is an unexpected adaptive response which attempts to offset, in part, the hematologic effects of iron deficiency.

RECOMMENDATIONS

These nutritional studies have been completed, and further investigations along these lines have been discontinued.

PUBLICATIONS

None

STUDY NO. 2 Effect of wounding on muscle metabolism

PROBLEM

Minimizing post-injury mobilization of functional muscle mass represents a significant problem in the treatment of trauma victims. Nutritional, hormonal, and pharmacological treatments designed to promote healing and preserve normal body composition need to be developed. The use of a greater percentage than normal (or even 100%) of the branched chain amino acids (BCAA) in post-traumatic intravenous nutritional support has been suggested by some investigators as a means to minimize catabolism and promote protein synthesis. There has been no direct experimental evidence to support this contention; thus, the fate of the BCAA in the wounded versus control animals seemed to be an important step in assessment of the efficacy of high BCAA therapy.

Response of Muscle to Injury (Cont)

RESULTS AND DISCUSSION OF RESULTS

Rats were wounded by intramuscular injection of 0.5% λ-carrageenan into the hindlimbs. This has been shown to produce a lesion characterized by myolysis, increased glucose clearance and lactate production, and increased release of BCAA from the wounded tissue. Six days following injury, branched chain amino acid transaminase (BCAAT) activity was assayed in homogenates of muscle from wounded and intact animals, and is reported as μmol leucine degraded/hr/mg protein. Control animals had a mean BCAAT activity of 56.5 ± 4.3 while wounded animals had a mean activity of 28.2 ± 1.8. It appeared that wounded tissue was less able to metabolize BCAA. Intracellular concentrations of amino acids in the tissue were calculated from tissue and whole blood amino acid values. The results are 158.7 ± 23.6, 74.0 ± 9.6, and 350.3 ± 2.5 for leucine, isoleucine, and valine, respectively, for control animals. Values for the wounded animals were 491.2 ± 36.0, 240.0 ± 26.0, and 761.8 ± 48.6. Intracellular concentrations are approximately double the control values in the injured tissue, certainly not what one might expect if the wounded tissue indeed had a higher BCAAT activity. In vivo BCAA oxidation was estimated by trapping $^{14}CO_2$ in the expired air from intact and wounded rats which had been injected with a tracer dose of ^{14}C-U-leucine. Non-wounded rats expired 12.1 ± .1% of their injected dose within 6 hours; the value for wounded rats was similar, 11.4 ± .2%. There was clearly no indication that whole body utilization was different between the groups. A possible mechanism to explain the increased BCAA efflux from the hindlimb during perfusion is that they build up intracellularly due to the decreased degradative capacity and are released in the greater amount based simply on equilibrium considerations.

CONCLUSIONS

The often-heard statement that BCAA utilization is higher in the traumatized individual can be questioned. It appears possible that these amino acids may be concentrated in the cell, but not necessarily oxidized. Other methods of inflicting a reproducible injury must be developed in order to determine if the observed results are unique to the carrageenan injection-induced wound.

RECOMMENDATIONS

This work should be continued. It will be possible to formulate improved methods and strategies of immediate posttrauma and postsurgery metabolic support if the basic mechanisms responsible for nutrient partitioning are identified.

PUBLICATIONS

None

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 021 A Porcine Model for Studies in Combat-Related Trauma

The following investigations were conducted under this work unit:

STUDY NO. 1 Normal physiological and biochemical values for the domestic pig (Sus scrofa)

STUDY NO. 2 Blood gas, acid-base, and hemodynamic responses of the conscious pig to hemorrhage

STUDY NO. 1. The blood gas and acid-base status of arterial and venous blood were characterized in 15 young pigs maintained under steady-state ventilatory conditions while anesthetized with nitrous oxide. In addition, population characteristics for all of the major cations and anions of arterial blood were determined. The blood biochemistry of the pig was remarkably similar to that of humans when ventilation was regulated to maintain an arterial pH of 7.40 and a P_{O_2} of 100 torr. The only major exceptions were a slightly higher bicarbonate buffer capacity and a lower arterial oxygen content. Similar studies were conducted over a 7-hour period in 31 conscious recumbent pigs monitored by means of chronically implanted arterial catheters. Again, the blood biochemical similarities to humans were demonstrated. Hemodynamic measurements in these animals revealed somewhat higher heart rates and arterial pressures than those seen in unanesthetized men.

STUDY NO. 2. Procedures were developed to anesthetize pigs for experimental surgery and for the placement of chronic arterial and venous catheters which would allow long-term monitoring of blood chemistry and physiological variables in the conscious animal. Subsequently, groups of 6 conscious recumbent animals were subjected to 30 or 50% blood loss over a 1-hour period and were monitored for arterial blood gas and acid-base changes and for hemodynamic modifications before, during, and for 5 hours after the hemorrhagic episode. In the 50% blood loss group, but not the 30% group, hemorrhage was associated with a transient increase in heart rate, a slight decrease in arterial pH and moderate decreases in P_{CO_2}, $[HCO_3^-]$, and base excess concentration. These changes were attributable in part to hyperventilation and in part to inadequate tissue perfusion leading to lactic acid production. During the recovery period, all of the foregoing changes reverted toward normal values over a 5-hour period. The reversion was attributable to the combined effects of tissue fluid mobilization to restore blood volume and a progressive rise in tissue perfusion as evidenced by an increase in heart rate. On the basis of these measurements, the pig is similar to the conscious human in terms of its responses to severe blood loss.

AD-A122 728 ANNUAL RESEARCH PROGRESS REPORT FY 1980(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA J D MARSHALL 01 OCT 80 3/3

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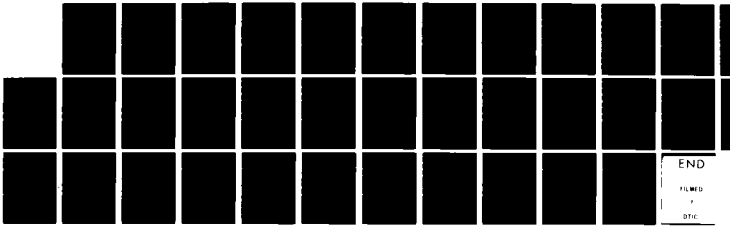

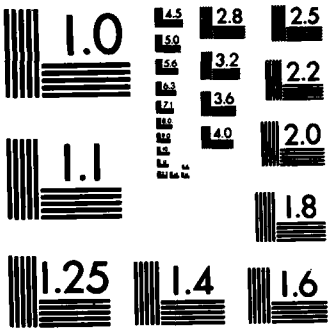

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 3369	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 07 15	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	091 APC HL14
b. [redacted]	62772A	3S162772A814	00	021
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)

(U) A Porcine Model for Studies in Combat-Related Trauma

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 012900 Physiology; 002300 Biochemistry; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	0.6	43
FISCAL YEAR CURRENT 81	1.5	50

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Hypovolemic Shock; (U) Swine; (U) Trauma; (U) Resuscitation; (U) Hemodynamics; (U) Metabolic Function

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) There is a distinct need for a large, nonprimate, animal model to conduct simulated studies of combat-related trauma, severe blood loss and consequent shock. From a scientific standpoint, the domestic pig would appear to be an attractive species for meeting this need, much more so than the commonly used mongrel dog. Use of the pig, however, has been hampered by a lack of knowledge about his normal physiological and biochemical characteristics and the impact thereon of simulated combat injuries. This information is needed to more accurately describe such injuries and to foster rational development of improved treatment modalities.

24. (U) Surgical and technical procedures will be developed to study the physiological and biochemical characteristics of the conscious, unencumbered animal. The effects of injuries and severe blood loss, such as seen in the combat environment, will be described and the effects of conventional and innovative treatment modalities will be evaluated.

25. (U) 79 10 - 80 09 Under steady-state ventilatory conditions, 15 anesthetized pigs were characterized in terms of arterial electrolytes, blood gas and acid-base status. Procedures were developed for the chronic implantation of arterial and venous catheters in conscious animals; patency was maintained for periods up to 6 weeks. The arterial blood gas and acid-base status, the arterial systolic, diastolic, pulse, and mean pressures, and the heart rate and hematocrit of 31 conscious, recumbent pigs were characterized over 7-hour periods of study. Groups of 6 conscious, recumbent pigs were subjected to 30 or 50% blood loss over a 1-hour period and were evaluated for arterial blood gas and acid-base changes as well as for hemodynamic variations before, during, and for 5 hours after hemorrhage. The conscious pig was found to be an excellent animal model to study the effects of, and treatment for, severe blood loss such as that seen in combat casualties.

*Available to contractors upon originator's approval.

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BODY OF REPORT

WORK UNIT NO.	021	A Porcine Model for Studies in Combat-Related Trauma
STUDY NO.	1	Normal physiological and biochemical values for the domestic pig (Sus scrofa)

PROBLEM

In the past, and at the present time, mongrel dogs have served as the predominant large animal species for medically oriented research on problems of combat-related trauma. Such usage is largely attributable to tradition and to the availability of dogs at local pounds and animal shelters. In recent years, however, the use of dogs in medical research has come under increasing criticism by scientists because they exhibit functional characteristics that are not seen in humans. The domestic pig, consequently, is becoming an attractive alternative to the dog as a large animal model for human oriented research. Pigs are readily available in all parts of the country and can be acquired in a variety of ages, sizes, and genetic backgrounds. Between-animal functional variances, therefore, are usually far less than those seen in mongrel dogs. But more important than these considerations, available information shows the pig to be far superior to the dog in terms of his physiological and biochemical similarities to man. In many research situations these similarities should allow substitution of pigs for nonhuman primates, hence conserving an expensive and rapidly diminishing laboratory animal resource. A major impediment to more extensive use of pigs in combat injury and other medical research projects is a lack of detailed knowledge about the population characteristics for certain key aspects of normal porcine physiology and biochemistry. Without this knowledge, rational experimental work involving pigs cannot be designed, nor can meaningful information about the functional changes associated with simulated combat injuries be obtained. It is to these problems that the experiments conducted under this study are directed.

RESULTS AND DISCUSSION OF RESULTS

Two experiments were conducted. The first was concerned with delineation of population characteristics of arterial electrolytes, blood gas, and acid-base status of young domestic pigs anesthetized with nitrous oxide and maintained under steady-state ventilatory conditions similar to those used in human surgical procedures. Fifteen animals with an average body weight of 29 kg were thus evaluated in terms of arterial plasma concentrations for sodium, potassium, calcium, magnesium, chloride, bicarbonate, phosphate, albuminate, globulinate, and lactate as well as for arterial and venous pH, P_{O_2}, P_{CO_2}, S_{O_2}, C_{O_2} (HCO_3^-), and base excess concentration at the end of a one-half hour period of

ventilatory stabilization during which arterial pH was established and maintained at pH 7.40 and P_{O_2} at 100 torr. Under these conditions, the blood characteristics of pigs appeared remarkably similar to those of humans. The only exceptions were a slightly higher bicarbonate buffer capacity and a slightly lower oxygen carrying capacity.

The second experiment was designed to delineate the population characteristics of conscious recumbent pigs in terms of arterial hemodynamics, arterial blood gas, and acid-base status. Thirty-one pigs with an average weight of about 25 kg were studied. They were brought into the laboratory in a portable cage and allowed a one-hour period of voluntary recumbency to achieve a metabolic steady-state. This was verified by serial blood gas and acid-base measurements made on arterial blood samples obtained by means of a chronically implanted catheter (see Study No. 2). At the end of this period and at one-hour intervals over a 6-hour period thereafter, arterial blood samples were drawn and characterized in terms of pH, P_{CO_2}, P_{O_2}, $[HCO_3^-]$, and base excess concentration. At these same time points heart rate, mean arterial pressure, and systolic, diastolic, and pulse pressures were recorded. Conscious pigs had a somewhat higher arterial pH and bicarbonate buffer capacity but a somewhat lower P_{O_2} than commonly seen in young conscious men. Pigs also had higher heart rates and arterial pressure values than humans. The only statistically significant diurnal variations were a slight increase in mean arterial pressure and slight decreases in arterial P_{O_2} and base excess concentration.

CONCLUSIONS

In terms of the blood biochemical and hemodynamic values obtained in this study, the domestic pig appears to be an excellent animal model for the study of combat-related injuries. In most respects its physiological and biochemical characteristics were remarkably similar to those of humans. The ease with which the conscious recumbent pig can be studied should allow the collection of experimental data which are directly relevant to the functional characterization and treatment of soldiers injured on the battlefield.

RECOMMENDATIONS

Additional physiological and biochemical characterizations of the normal, particularly the conscious pig, should include total body, regional, and tissue oxygen delivery, arterial and venous metabolite and hormone levels, renal function values, and the regulatory characteristics of various physiologic systems.

PUBLICATIONS

None

A Porcine Model for Studies in Combat-Related Trauma (Cont)

STUDY NO. 2 Blood gas, acid-base, and hemodynamic responses of the conscious pig to hemorrhage

PROBLEM

Virtually all previous studies of the physiology and biochemistry of hemorrhage and resultant hypovolemic shock have been conducted in anesthetized animals. Rarely does one see investigations utilizing conscious animals. In addition, the majority of large animal studies have been conducted with canine models. These studies, in general, suffer from two major deficiencies. One, combat injuries rarely, if ever, occur in anesthetized soldiers and it is a well-established medical fact that anesthetic agents seriously modify many of the normal physiological and biochemical responses to severe injury and blood loss. Secondly, in terms of many highly pertinent functional variables, the dog is not a good model for characterizing responses to severe hemorrhage so often seen on the battlefield. The applicability of such experimental information to the combat-injured soldier is critical to the rational development of effective medical treatment procedures at front line positions.

The domestic pig, in terms of its known functional characteristics, appears superior to the dog as an animal model for physiological and biochemical studies which are relevant to humans injured in combat. The pig, furthermore, can be readily studied under conscious unencumbered conditions in the laboratory. But, it is only in recent years that medical researchers have started to use the pig for studies of hemorrhage and shock, and even in these few instances virtually no experimental work has involved conscious animals. The present study, therefore, was designed to develop surgical procedures for monitoring the functional characteristics of conscious pigs over extended periods of time and to collect data on physiological and biochemical responses to severe blood loss.

RESULTS AND DISCUSSION OF RESULTS

The responses of young domestic pigs to a variety of anesthetic agents were evaluated in terms of their impact on blood gas and acid base status and their utility for routine experimental surgical procedures. The most reliable current procedure includes preanesthetic, intramuscular injections of 0.02 mg/kg atropine, 0.5 mg/kg ketamine HCl, and 0.5 mg/kg xyalazine HCl while the animal is confined to a portable carrying cage. Anesthesia is then induced with a halothane in oxygen mixture administered by a mask placed over the snout. Finally, during surgery anesthesia is maintained with a halothane-oxygen mixture administered by ventilator through a cuffed endotracheal tube. The only adverse effects noted with this anesthetic regimen was that an

occasional animal, perhaps 1 in 25, developed malignant hyperthermia, apparently in response to halothane.

Procedures for the chronic emplacement of arterial and venous catheters were developed and evaluated. The catheters consisted of an intravascular silicon rubber component and an extravascular tygon component. Such compound catheters were designed to minimize intravascular clotting problems, yet to maintain the desired physical characteristics needed for long-term physiological measurements. The tygon portion of the catheter was tunneled under the skin to the back of the neck, where it was exteriorized and capped with an appropriate stub adaptor fitted with a plastic/rubber intermittent infusion plug. These lightweight exterior portions allowed ready access for physiologic measurements and blood sampling. The infusion plug allowed flushing of the catheter with heparinized saline as needed to maintain patency, yet minimized the introduction of infective organisms. When not in use, cleanliness of the exterior portions was maintained by a Velcro patch permanently sutured to the skin. Patency of these catheters has been maintained for as long as 6 weeks and consistently for 1 to 2 weeks when flushed with heparin at weekly intervals. Failure, when it occurred, was usually due to kinking which in turn was due to the pig's outgrowing the length of catheter implanted.

Groups of 6 conscious recumbent pigs with chronically implanted arterial catheters were subjected to 30 and 50% blood loss over a one-hour period and were evaluated for arterial acid-base and blood gas changes as well as for hemodynamic variations before, during, and for 5 hours after the hemorrhagic episode. All animals survived these treatments. During hemorrhage, both groups exhibited reduced values for arterial mean, systolic, and diastolic pressure; the effects were significantly more pronounced in the 50% hemorrhage group. The 50% group, but not the 30% group, showed a transient rise in heart rate during the early stages of blood loss. Subnormal heart rates were observed subsequent to hemorrhage, particularly in the 50% group. During the 5-hour recovery period progressive tachycardia was recorded, with the effect being more pronounced in the 50% group. Hemorrhage in the 50% group, but not the 30% group, was associated with a slight but significant decrease in arterial pH and moderate decreases in P_{CO2}, $[HCO_3^-]$, and base excess concentration. These effects were attributable in part to hyperventilation and in part to inadequate tissue perfusion with resultant lactic acid formation. During the recovery period the foregoing alterations were reversed and normal blood chemical characteristics were approached at the end of 5 hours. This was apparently attributable to the mobilization of tissue fluid to re-establish blood volume since a progressive decrease in hematocrit was observed during the recovery period.

CONCLUSIONS

The foregoing experiments have demonstrated that the effects of severe blood loss in the conscious animal can be readily studied in the domestic pig. For the most part, the physiological and biochemical changes associated with a hemorrhagic episode appeared to be similar to those reported for conscious humans. The pig may be more tolerant of blood loss than the human, but this is not known with certainty since comparable experiments cannot be performed on human subjects.

RECOMMENDATIONS

If the pig is more tolerant to hemorrhage than the human, the physiological factors imparting this advantage need to be identified. Such information would be of value in the design of treatment modalities for forward use in battlefield situations. In addition, the physiology of hemorrhage and resultant hypovolemic shock in swine needs to be described in terms of total body and localized tissue oxygen delivery, consequences of local ischemia on tissue and organ functions, and responsiveness of the species to conventional or innovative treatment procedures for hypovolemic shock.

PUBLICATIONS

1. HANNON, J.P. Microdetermination of Sucrose in Plasma with the Anthrone Reagent. Report No. 80. San Francisco, California: Letterman Army Institute of Research, November 1979

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 2374	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AB	092 APC HL15
b. [redacted]	62772A	3S162772A814	00	022
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Pharmacological Stabilization of the Combat Casualty

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

008800 Life Support; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	1.2	41
	CURRENT 81	4.5	106

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ASSOCIATE INVESTIGATORS

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NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Resuscitation; (U) Irreversibility; (U) Hemorrhagic Shock; (U) Critical Organ Failure

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Optimal management of combat casualties requires expeditious evacuation to treatment facilities capable of providing definitive surgical care. Since battlefield conditions in future wars may preclude rapid air and ground evacuation, it is desirable to develop nonsurgical means, capable of being applied by combat medics, that will delay the pathophysiological consequences of neglected wounds. Recently published work suggests several pharmacological interventions that may be of value: 1) blockade of β-endorphin opiate receptors (nalaxone), 2) interference with the formation of vasoactive prostaglandins (indomethacin), and 3) provision of high energy substrates capable of being metabolized more efficaciously than glucose (fructose-1,6-diphosphate).

24. (U) A small animal hemorrhagic shock model using rats will be developed to investigate the feasibility of formulating an "antishock cocktail."

25. (U) 79 10 - 80 09 A fixed volume withdrawal hemorrhagic shock model using conscious rats has been developed. Twenty-five percent of the animals survive beyond six hours following onset of hemorrhage. None of the following drugs, when given after the initial hemorrhage, alter survival: nalaxone (1 mg/kg), benadryl (1 mg/kg), imidazole (1 mg/kg), fructose-1,6-diphosphate (50 mg IV). Survival following infusion of Ringer's lactate is 75%. Work is in progress evaluating different doses and schedules for administration.

*Available to contractors upon originator's approval.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 FOR ARMY USE ARE OBSOLETE

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 022 Pharmacological Stabilization of the Combat Casualty

A fixed volume withdrawal hemorrhagic shock model has been developed using conscious rats for the purpose of studying drug therapy as a substitute for blood replacement in exsanguination. Twenty-five percent of the untreated animals survive. Naloxone (1 mg/kg), diphenhydramine (1 mg/kg), fructose-1,6-diphosphate (200 mg), and imidazole (1 mg/kg) have been studied; these drugs did not change the survival rate. Seventy-five percent of the exsanguinated animals survived when they were given Ringer's lactate. Different drugs and new dose schedules for previously tested drugs are being investigated.

BODY OF REPORT

WORK UNIT NO. 022 Pharmacological Stabilization of the Combat Casualty

PROBLEM

Optimal management of combat casualties requires that evacuation be prompt from the battlefield to facilities capable of providing definitive surgical care. Unfortunately, there is no certainty that rapid evacuation as practiced in former wars will be possible in the future. The development of nonsurgical interventions which might help certain categories of combat casualties to survive is clearly indicated. A number of publications have appeared recently which suggest that the outcome in various animal shock models can be favorably influenced by drug therapy. Among the proposed antishock drugs are: 1) diphenhydramine for H^1 histamine blockade in hemorrhagic shock; 2) nalaxone for beta-endorphin opiate receptor blockade in hemorrhagic and endotoxin shock; 3) indomethacin and imidazole for inhibition of prostaglandin synthesis in hemorrhagic and endotoxin shock; 4) lidocaine in endotoxin shock; 5) fructose-1,6-diphosphate, an alternative source of high energy phosphate in hemorrhagic and endotoxin shock; 6) blockage of angiotensin-converting enzyme in hemorrhagic shock; 7) prostacyclin in endotoxin shock; and 8) $ATP \cdot MgCl_2$ in hemorrhagic shock. Although published data frequently show quite a remarkable improvement in survival rates (e.g., survival increases from 0% to 100% when rats are given diphenhydramine prior to being subjected to hemorrhage), the relevance of many of the shock models to the treatment of the bleeding soldier is not always obvious. The purpose of this study is to investigate the effectiveness of various antishock drugs by utilizing a model which has been designed to simulate battlefield trauma. A fixed volume withdrawal hemorrhagic shock model using conscious rats has been developed. Catheters are placed into the jugular vein and carotid artery of anesthetized 350-400 g rats. The next day, while awake, 50% of the rat's calculated blood volume is removed over a period of one hour. Fifteen minutes after the start of hemorrhage the rat is given an "antishock" drug. Survival is assessed at 6 hours.

RESULTS AND DISCUSSION OF RESULTS

Twenty-five percent of the untreated or control animals survived. Naloxone (1 mg/kg), diphenhydramine (1 mg/kg), fructose-1,6-diphosphate (200 mg), and imidazole (1 mg/kg) have been studied; none of these agents changed the survival rate. Preliminary data suggested that naloxone (2 mg/kg) might increase the survival rate. The fructose-1,6-diphosphate data may be invalid because of unsuspected contamination of the commercially available preparation. Seventy-five percent of the exsanguinated animals survived when they were given Ringer's lactate.

Pharmacological Stabilization of the Combat Casualty (Cont)

CONCLUSIONS

Further work is necessary to evaluate the potential value of antishock drugs in a field setting.

RECOMMENDATIONS

A number of drugs remain to be evaluated. We must evaluate different doses and schedules for administration for the previously tested drugs.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMM.	REPORT CONTROL SYMBOL
DAOG 2387	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	095 APC HL16
b. [redacted]	62772A	3S162772A814	00	023
c. [redacted]	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)*

(U) Metabolic Support Following Combat Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 008800 Life Support; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	1.5	61
FISCAL YEAR CURRENT 81	0.6	20

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NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Body Compositional Change; (U) Wound Healing; (U) Military Trauma; (U) Parenteral Nutrition; (U) Animal Model

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The intravenous administration of crystalline amino acid solutions has been shown to decrease negative nitrogen balance, maximize protein flux and maintain immune competence better than isocaloric dextrose infusions alone in the post-trauma post-surgical patient unable to eat. The objective of this study is to determine if the non-essential amino acids (NEAA) can be replaced by glucose without a detrimental effect on the animal's N balance or body composition. This would result in lower cost as well as reduced BUN and ketosis.

24. (U) Rats were maintained orally or intravenously on 20% of their daily caloric requirement for 4-5 days. The use of all amino acids (AA), all glucose (GLU), and glucose + essential amino acids (EAA) was compared by monitoring changes in body composition, body weight, nitrogen balance, and circulating levels of urea, glucose, amino acids, and ketone bodies.

25. (U) 79 10 - 80 09 It was found that all the NEAA could be replaced by dextrose with no deleterious effect on any of the parameters measured if the ratio of EAA was optimal for the animal. If the ratio of EAA was not close to the rat's requirement, the inclusion of NEAA was required to achieve results equal to that resulting from amino acids alone. At these low levels of caloric intake, far below that required for growth or maintenance of the animal, there was no difference in nutrient utilization between intravenously- and orally-fed animals.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3S162772A814 Military Trauma and Resuscitation

WORK UNIT NO. 023 Metabolic Support Following Combat Injury

The following investigation was conducted under this work unit:

STUDY NO. 1 Isotonic dextrose and essential amino acids as hypocaloric short-term metabolic support

Four experiments were conducted to compare the effects of semistarvation regimens of isocaloric combinations of glucose and amino acids on body composition and selected metabolic parameters of adult rats. Diets were administered either orally or intravenously at the rate of 20% of the daily caloric requirement of the rat. In all cases there was an improvement in nitrogen balance when all the calories were supplied as amino acids when compared to glucose alone. Experiment 2 resulted in equal nitrogen balance when any of the calories were supplied as amino acids when compared to glucose. Experiments 3 and 4 showed equal nitrogen balance when half the amino acids were replaced by glucose (compared to amino acid alone), but replacing only the nonessential amino acids did not improve nitrogen balance when compared to glucose alone. The differences in the ratios of the amino acids of the diets may have been responsible for the differences observed. There was no apparent difference in the response of the animals whether the diets were administered orally or intravenously, and animals from all treatments showed similar weight loss and body composition changes following the dietary treatments. It is concluded that replacement with glucose of at least one-half the calories of an all-amino-acid-hypocaloric diet does not adversely affect body composition, weight change, or nitrogen balance but may reduce blood urea nitrogen (BUN), ketosis, and cost of the diet.

BODY OF REPORT

WORK UNIT NO.	023	Metabolic Support Following Combat Injury
STUDY NO.	1	Isotonic dextrose and essential amino acids as hypocaloric short-term metabolic support

PROBLEM

Maintenance of normal body composition requires adequate nutrient intake. Loss of lean body mass, and the associated increase in morbidity and mortality, is a significant problem in the treatment of the combat-injured individual deprived of oral intake. Efforts of surgical metabolism research have been directed at providing some percentage of the required nutrients as immediate intravenous support to minimize the deleterious impact of semistarvation in the already-compromised injured individual. Isotonic dextrose (5%) constitutes the most common form of posttrauma, postsurgical support. Many isotonic amino acids administered intravenously improve nitrogen balance. Plasma protein and immunocompetent status may be improved also.

It is my hypothesis that the beneficial effect of the administration of all amino acids was the provision of essential amino acids, which animals are unable to synthesize. The others, so-called nonessential amino acids, can be synthesized within the animal by using endogenous carbohydrate intermediates and nitrogen sources. If this is true, then it should be possible to replace the nonessential amino acids (NEAA) with dextrose but maintain equal protein and nitrogen status. If glucose plus essential amino acids (GLU+EAA) are administered, it should be possible to gain the benefits of all-amino-acid therapy without the elevated blood urea nitrogen (BUN) and ketosis associated with all-amino-acid therapy. The initial experiments consisted of studying the effects of isocaloric exchange of glucose and various amino acids on nitrogen balance and body composition in the laboratory rat.

RESULTS AND DISCUSSION OF RESULTS

Male Sprague Dawley rats (280-380 g) were used for all 4 experiments. Rats to be fed orally were fasted overnight and fed the hypocaloric solutions in 3 equal aliquots at 8-hour intervals. Total urine and fecal collections were made throughout the experiment. At the termination of the experiment, animals were anesthetized, bled via cardiac puncture to obtain samples for analysis, and killed.

In the second experiment, fasted rats were prepared for intravenous feeding by external jugular catheterization. Solutions were delivered aseptically through the central venous catheter by means of a constant

infusion pump. On the morning of the fifth day of the experiment, the infusion was stopped 1 to 2 hours before killing the animals. They were anesthetized, weighed, and bled via cardiac puncture.

Differences in nitrogen balance and body composition among rats consuming glucose, amino acids, and three combinations thereof were examined using one amino acid formulation for the 2 experiments. First, a group receiving no calories was included to assess the difference in the metabolic response to total- versus semi-starvation. No mortality was observed except in the starvation group (2 of 6 died, Day 4). The results of this experiment show there was a significant improvement in nitrogen balance when any calories were provided, and a further improvement when all or some of the calories were in the form of amino acids when compared to glucose. There were no differences in total body water, fat, or protein among any of the groups receiving calories, although there was less fat in the carcasses of the starved animals. Liver protein content was similar for all dietary regimens.

The third experiment compared the effect of a commercially available EAA source on the same parameters as the first two experiments. Using this formulation (Abbott's Renal^{TM} plus arginine), a mixture of the EAA and NEAA was required to produce nitrogen balance significantly better than that obtained using glucose alone. Weight loss and plasma glucose were unaffected by the alterations in diet composition. Plasma hydroxybutyrate levels were significantly lower in the glucose and GLU+5% EAA than the all-amino-acid group, although the nitrogen excretion was the same. There were few striking changes in whole blood amino acid levels brought about by differences in the diets. Alanine was present in greater amounts in the animals receiving glucose alone or glucose + 5% EAA when compared to all-amino-acid diet. The basic amino acids were also higher in the groups receiving primarily glucose. Tryptophan levels were very low in all groups, although they were highest in the all-amino-acid group.

The fourth experiment compared the effects of intravenous administration of the same diets used in Experiment 3. Live weight loss and changes in body composition were unaffected by diet composition in this experiment. Nitrogen balance was improved when some or all of the calories were supplied as a mixture of EAA and NEAA when compared to glucose. In this experiment, BUN did correspond with amino acid content of the diet, although the differences were unremarkable. Plasma acetoacetate was five to eight times the normal for all treatments. Amino acid patterns were similar to those seen in Experiment 3, although glutamine levels were higher and the branched chain amino acids were depressed in the animals receiving glucose alone. Tryptophan levels were higher than in the third experiment, which indicate that absorption from the gut was inefficient or that this amino acid was metabolized by the gastrointestinal tract in the orally fed animals.

CONCLUSIONS

The present studies indicate that development of distinct formulations of amino acids for the semistarving patient, distinct from those used during total parenteral nutrition, may be beneficial. It has been shown that glucose does not necessarily detract from the "nitrogen-sparing" qualities of hypocaloric amino acid administration, although the use of glucose seems to be determined by the amino acid composition of the rest of the diet. Amino acid patterns in animals fed or infused with hypocaloric diets showed definite deficiencies of tryptophan and tyrosine, which indicate the possibility that these may be limiting the utilization of the other amino acids. Use of commercially available crystalline amino acid mixtures was not optimal for the rat, apparently due to the quantitative differences in amino acid requirements and metabolic differences between man and rat. It appears likely that further study will result in our ability to supply the "critical" amino acids in a much less nitrogen-dense formula that is currently recommended.

RECOMMENDATIONS

These studies should be continued. Ways to incorporate greater quantities of the apparently limiting amino acids should be examined. Studies similar to those just described need to be done in animals following shock or injury. Attempts should be made to correlate changes in body composition and nitrogen balance with various measures of physiological response.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA0G2348	2. DATE OF SUMMARY* 80 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 80 08 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8a. DISP'N INSTR'N NL	8b. SPECIFIC DATA - CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A875	CD	301 APC FL 04
b. [redacted]	62780A	3E162780A843	00	051
c. [redacted]	STOG	80-7.2:4		

11. TITLE (Procede with Security Classification Code)*

(U) Skin Decontamination Technology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003200 CBR Warfare; 004900 Defense; 017100 Weapons Effects

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	1.9	107
FISCAL YEAR CURRENT 81	6.1	282

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Models; (U) Simulants; (U) Chemical Defense; (U) Dermal; (U) Decontamination; (U) Skin; (U) Cutaneous; (U) Nerve Agents; (U) Vesicants

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) On the modern battlefield, both conventional and chemical (CW) casualties may have sublethal amounts of agent on their skin. Decontaminants and decontamination systems are needed to protect patients from further insult and to protect medical personnel from secondary exposure while treating them. Methodologies to measure sublethal levels of agents and standardized model systems which can be used instead of humans, are needed for assessing degrees of contamination and efficacy of decontamination.

24. (U) Models will be developed and standardized to provide human-relevant data for skin decontamination studies. Quantitative and qualitative methods will be developed for determining which patients require decontamination and for assessing the efficacy of decontamination. Risks associated with CW agent exposure and decontamination will be assessed to aid in triage and treatment. Where possible, low hazard simulants of agents will be used to facilitate early investigation and promote safety. Key experiments and results will be validated with agents in facilities that meet surity requirements.

25. (U) 79 10 - 80 09. A bench model shower device developed at USAMBRDL and a LAIR permeability apparatus were used to determine the effects of pressure, detergent temperature, nozzle type, and wash time on removal of diethylmalonate (DEM) and thickened DEM (non-toxic physical-chemical simulants of soman (GD and thickened GD) from human skin. Force per unit area was the major factor affecting decontamination efficacy. Showering also enhanced percutaneous penetration of DEM. Studies were begun to determine the relevance of DEM data for simulating GD decontamination parameters by repeating key experiments with GD at USABML.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3E162780A843 Defense Against Chemical Warfare

WORK UNIT NO. 051 Skin Decontamination Technology

The following investigation has been conducted under this work unit during the past year:

STUDY NO. 1 In vitro determination of shower decontamination efficacy

An apparatus has been developed to study the efficacy of shower decontamination of skin in a controlled setting. This apparatus allows quantitative removal of chemicals from the skin surface. A bench model of the breadboard device was used in this study to determine the most important parameters in removal of diethylmalonate (DEM) and thickened DEM from skin. Force per unit area of the shower spray was the most important factor in removal of DEM with the USAMBRDL breadboard patient shower decontamination device. Showering also promoted percutaneous penetration of DEM. A collaborative study has been started with the U.S. Army Biomedical Laboratory (USABML) to determine if the same results will be obtained with nerve agent soman (GD).

BODY OF REPORT

WORK UNIT NO. 051 Skin Decontamination Technology

STUDY NO. 1 In vitro determination of shower decontamination efficacy

PROBLEM

In a battlefield environment where chemical warfare (CW) agents are used, conventionally wounded casualties may also be chemically contaminated. CBR protective clothing restricts the ability of medical personnel to provide necessary treatment to patients and, therefore, medical personnel must operate in a shirt sleeve environment when caring for chemically contaminated patients on litters. These casualties must be decontaminated before they receive medical treatment for wounds. Decontamination protects medical personnel by preventing secondary exposure to detrimental levels of chemical agents. Decontamination should also be performed in such a manner that it does not further compromise the patient's condition. Designers do not have sufficient information on nonambulatory casualty decontamination to construct a prototype device for deployment and installation. To obtain the necessary information, a breadboard model (an experimental item of hardware fabricated during the conceptual phase to reduce technological uncertainty, prove feasibility, and provide realistic cost estimates) of a decontamination device has been fabricated by the U.S. Army Medical Bioengineering Research and Development Laboratory. The measurements of variables functioning in the breadboard device (water pressure, soap concentration, water temperature, nozzle type, wash time) were not known. In this study we assessed, in vitro, the effect of these variables on the removal of nontoxic agent simulants (diethylmalonate and thickened diethylmalonate were used as simulants for soman and thickened soman, respectively) from the surface of excised pig and human skin. To do this, a decontamination bench model was used to simulate the function of the breadboard decontamination device. The bench model has only a single stationary nozzle.

RESULTS AND DISCUSSION OF RESULTS

The force per unit area exerted by the shower on the skin surface is the major variable responsible for differences in decontamination efficacy. Time may play a more important role at low force ($2.1X10^3$ $dyne/cm^2$) per unit area, but time is unimportant at high force ($8.3X 10^3 dyne/cm^2$) per unit area. Differences in nozzle type and the addition of Triton X-100 to the decontamination fluid generally did not

influence decontamination efficacy. Showering always increased the mean percutaneous penetration of the simulants during a 15-minute period as compared to no showering. Simulants studied were selected on the basis of physical properties only. The significance of increased percutaneous penetration will not be known until comparative permeability data between the simulants and agents are available.

CONCLUSIONS

For the simulants tested, the mechanism of cleaning is probably mechanical. The force per unit area variable had the greatest impact on decontamination effectiveness. Decontamination solution temperature and the presence of Triton X-100 did not greatly influence decontamination efficacy. Percutaneous penetration was increased with showering; significance of this result will not be known until comparative permeability studies between the simulants and agents are done.

RECOMMENDATIONS

A study entitled "Percutaneous penetration of the nerve agent, soman, assessment of diethylmalonate as a simulant for soman," has been initiated and is programmed to be completed by our laboratory in cooperation with the U.S. Army Biomedical Laboratory.

PUBLICATIONS

REIFENRATH, W.G. Shower Decontamination Efficacy. In Vitro Determination. Final Report. Institute Report No. 86. Presidio of San Francisco, California: Letterman Army Institute of Research, August 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OG 2372	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	H.TERMINATION	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62780A	3E162780A843	00	053 APC 505H
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Care of the Chemical Casualty

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012600 Pharmacology; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	80 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	1.5	59
FISCAL YEAR CURRENT 81	0.0	00

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: POC: DA

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Delayed Neurotoxic Syndrome; (U) DFP; (U) Organophosphate; (U) Combat Chemical Casualty; (U) Chronic Toxicity; (U) Decontamination

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Delayed neurotoxic syndrome (DNTS) may occur after exposure to nerve agents used in chemical warfare or after exposure to lubricants that contain tri-aryl phosphates. The objectives of these studies are to find protective chemicals for nullifying the neurotoxic esterase (NTE) activity which causes this syndrome and to produce a decontamination solution that can be used on personnel who are also suffering from wounds caused by conventional weapons. Techniques developed during this research will also provide a method for screening lubricants for this type of toxicity and providing agents beneficial in preventing it.

24. (U) A suitable animal model and an NTE assay will be developed for studying delayed neurotoxicity and for screening potential protective compounds. Methods will be developed to isolate, stabilize and assay DFP-hydrolyzing enzyme to characterize an in vitro system capable of detoxifying an organophosphate.

25. (U) 79 10 - 80 09. In collaboration with the Division of Biorheology, the rat was evaluated as a mammalian model of DNTS. As measured in various psychomotor tasks, no differences were observed between experimental and control groups during the chronic phase of the experiment. Another animal model must be found. An assay using paraoxon as the substrate was established for DFPase. Thirteen resins were examined for efficacy in isolating and stabilizing DFP-hydrolyzing enzyme. No resin or elution conditions were effective, so use of sequential 2-resin systems should be considered. The risk of DNTS is real and should be addressed, but present priorities dictate termination of the work at this time to allow concentration of research efforts on prevention of acute chemical injuries.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3E162780A843 Defense Against Chemical Warfare

WORK UNIT NO. 053 Care of the Chemical Casualty

The following investigations were pursued under this work unit:

STUDY NO. 1 Prevention and care of the chronic phase of delayed neurotoxicity

STUDY NO. 2 Decontamination of the chemical casualty

STUDY NO. 1. In collaboration with the Division of Biorheology, rats were tested periodically to determine the onset and severity of delayed neurotoxic syndrome (DNTS). As measured by various psychomotor tasks, no difrerences were observed between any experimental and control groups during the chronic phase of the experiment.

STUDY NO. 2. Several resins and elution conditions were examined in terms of their ability to isolate and stabilize DFPase. During the course of this research, 13 different resins were studied. A combination 2-resin system remains to be evaluated.

BODY OF REPORT

WORK UNIT NO.	053	Care of the Chemical Casualty
STUDY NO.	1	Prevention and care of the chronic phase of delayed neurotoxicity

PROBLEM

Subsequent to the acute phase of poisoning with some organophosphates, a delayed toxic response may develop. In man this effect is observed 2 to 7 days following initial exposure. The delayed neurotoxic syndrome (DNTS) is characterized by ataxia and paralysis which results from a destruction of both peripheral and central components of the spinal nerves. Although various therapeutic agents exist to treat the acute phase of organophosphate poisoning no treatments are available for use in DNTS. The aim of this study is to establish a mammalian model for study of DNTS since none exists and subsequently to use the model to screen various possible therapeutic agents.

RESULTS AND DISCUSSION OF RESULTS

These experiments were conducted in conjunction with the Division of Biorheology. Due to considerations of personnel and available equipment the rat was evaluated as a mammalian model of DNTS. DFP was administered subcutaneously at weekly intervals at different dosage levels (1.0, 0.5, 0.25, 0.125 mg/kg) for a period of approximately 3 months. Various physiological and psychomotor outputs were measured including: active avoidance tasks, open-field behavior and roto-rod activity. No differences were found between rats treated with DFP and a control group injected with peanut oil.

CONCLUSIONS

The rat is highly resistant to the effects of repeated administration of DFP and therefore is not a useful model to study possible therapeutic agents against DNTS.

RECOMMENDATIONS

A study of the mouse as a model of DNTS should be initiated.

PUBLICATIONS

None

Care of the Chemical Casualty

STUDY NO. 2 Decontamination of the chemical casualty

PROBLEM

Current methods of decontaminating personnel exposed to nerve agents involve the use of chemicals which are deleterious in nature. A possible alternative means of decontamination is through the use of an enzyme capable of destroying chemical agents. The enzyme must be fixed to a physical matrix which would preserve stability and maximize activity of the enzyme. This study was aimed at finding an appropriate resin meeting these requirements.

RESULTS AND DISCUSSION OF RESULTS

An assay for DFP was established with paraoxon as the substrate. Paraoxon was selected because its hydrolysis can be observed spectrophotometrically. This follows from the fact that paraoxon contains a chromophore which absorbs light at 420 nm. The hydrolysis of DFP can only be followed manometrically, a technique not currently available in our laboratory. Although a distinct DFPase enzyme may exist separately from a paraoxon hydrolyzing enzyme, it is considered probable that the isolation of an enzyme capable of hydrolyzing the P-X bond would serve the purposes of the study. After establishing the assay, various resins were examined as a means of isolating and/or stabilizing an organophosphate hydrolyzing enzyme. Currently, of 13 resins fully or partially examined, no resin or elution conditions seems appropriate. The evaluation of a sequential 2-resin system remains to be evaluated.

CONCLUSIONS

Matrix systems studied so far are not suitable for the isolation and stabilization of the enzyme. This may be due to the fact that ion-exchange resins were used in the majority of instances.

RECOMMENDATIONS

Newer affinity binders should be examined.

PUBLICATIONS

None

APPENDIX A

LAIR PUBLICATIONS ACCESSIONED - 1980

INSTITUTE REPORTS

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81 BLANCHARD, J.M., G.M. WARD, H.J. KRZYWICKI, and J.E. CANHAM. A visual appraisal method for estimating body composition in humans. November 1979

82 STAMPER, D.A., P.A. O'MARA, E.S. BEATRICE, and D.J. LUND. Tracking performance with a viscous-damped mount under simulated conditions of varied ambient light levels and target velocities. January 1980

83 TREVINO, G.S., J.H. SKALA, R.S. DEMAREE, J.G. MILLER, B.V. SANDERS, T.A. O'DONNELL, and J.E. CANHAM. Nutrition studies in military German shepherds consuming three commercial rations for thirty-five months with various levels of physical activity. June 1980

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TECHNICAL NOTES

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13 DONG, M.H., E.L. McGOWN, P.P. WARING, J.H. SKALA, and H.E. SAUBERLICH. Purification of transketolase from human erythrocytes. I. Using solvent denaturation as the initial step. July 1980

14 OMAYE, S.T., R.A. WIRTZ, L.S. GUTHERTZ, P.C. TAYLOR, and J.T. FRUIN. Hematologic and selected hepatic changes produced by substituted ρ-benzoquinones in the rat. July 1980

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80-001 RUTLEDGE, L.C., M.A. MOUSSA, B.L. ZELLER, and M.A. LAWSON. Field studies of reservoirs and vectors of sylvatic plague at Fort Hunter Liggett, California. J Med Entomol 15: 452-458, 1979

80-002 PRYSTOWSKY, S.D., A.M. ALLEN, R.W. SMITH, J.H. NONOMURA, R.B. ODOM, and W.A. AKERS. Allergic contact hypersensitivity to nickel, neomycin, ethylenedimine, and benzocaine; relationships between age, sex, history of exposure, and reactivity to standard patch tests and use tests in a general population. Arch Dermatol 115: 959-962, 1979

80-003 FRUIN, J.T., H.F. ALISHOUSE, and A. DUNCAN. A microbiological summary of food produced by the Central Food Preparation System at Ft. Lee, Virginia. In: Memorandum for Each Army Veterinary Corps Officer, Letter of November 1979, Subject: Army Veterinary Corps Information (6-79) 12 pp

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80-005 SAUBERLICH, H.E., Y.F. HERMAN, C.O. STEVENS, and R.H. HERMAN. Thiamin requirement of the adult human. Am J Clin Nutr 32: 2237-2248, 1979

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80-008 MILNE, D.B., D.D. SCHNAKENBERG, H.L. JOHNSON, and G.L. KUHL. Trace mineral intake of enlisted military personnel. J Am Diet Assoc 76: 41-44, 1980

80-009 ASKEW, E.W., G.L. DOHM, P.C. WEISER, R.L. HUSTON, and W.H. DOUB, JR. Supplemental dietary carnitine and lipid metabolism in exercising rats. Nutr Metab 24: 32-42, 1980

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80-011 HAGLER, L., R.I. COPPES, E.W. ASKEW, A.L. HECKER, and R.H. HERMAN. The influence of exercise and diet on myoglobin and metmyoglobin reductase in the rat. J Lab Clin Med 95: 222-230, 1980

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80-018 CABAUD, H.E., A. CHATTY, V. GILDENGORIN, and R.J. FELTMAN. Exercise effects on the strength of the rat anterior cruciate ligament. Am J Sports Med 8: 79-86, 1980

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80-025 BIKLE, D.D., E.W. ASKEW, D.T. ZOLOCK, R.L. MORRISSEY, and R.H. HERMAN. Calcium accumulation by chick intestinal mitochondria regulation by vitamin D_3 and 1,25-dihydroxy-vitamin D_3. Biochim Biophys Acta 598: 561-574, 1980

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80-027 CABAUD, H.E., W.G. RODKEY, and H.R. McCARROLL. Peripheral nerve injuries: Studies in higher nonhuman primates. J Hand Surg 5: 201-206, 1980

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80-032 FRUIN, J.T., C.D. KUZDAS, and L.S. GUTHERTZ. Mutagenicity studies with irradiated meats. (Abstract No. 20 for Food Irradiation Poster Session) In: Volume of Abstracts, 26th European Meeting of Meat Research Workers, International Meat Research Congress, (Colorado Springs, Colorado, 31 August - 5 September 1980)

80-033 MOORE, G.L., M.E. LEDFORD, and C.C. PECK. The in vitro evaluation of modifications in CPD-adenine anticoagulated-preserved blood at various hematocrits. Transfusion 20: 419-426, 1980

LAIR Publications Accessioned - 1980

80-034 BOLIN, R.B., B.A. CHENEY, O.A. SIMPLICIANO, and C.C. PECK. *In vitro* evaluation of platelets stored in CPD-adenine formulations. Transfusion 20: 409-418, 1980

APPENDIX B

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M.A. (Univ. of Colorado)

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Ph.D. (Univ. of Illinois)

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